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CORTICOTROPIC ACTIVITY OF HUMAN BLOOD*

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PREVIOUS workers in this laboratory have investigated the presence of corticotropin in the blood and urine of man. Contrary to earlier reports, no corticotropic activity could be consistently demonstrated in the urine of healthy persons and of patients with a variety of disorders [Locke, Albert and Kepler (1)].[1] However, corticotropin was consistently found in the serum of patients with untreated Addison's disease, but was not found in the serum of normal persons or of those with Cushing's syndrome [Taylor, Albert and Sprague (2)]. Since our two reports, numerous publications dealing with corticotropic activity of the blood in normal and in pathologic states have appeared. These publications report extremely variable and contradictory results. For this reason, and because our interest in the problem has continued, it seemed worth while to report data obtained since the publication of Taylor and associates (2) and to review the present status of assays for corticotropin in human blood.

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* Some of the results represent those in a thesis submitted by Dr. Upson to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine. Dr. Upson's present address is 2508 Auburn Ave., Cincinnati, Ohio.

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[1] Note added in proof. The recent paper by Rubin *et al.* (*J. Clin. Endocrinol. & Metab.* **14**: 154 (Feb.) 1954) reopens the question of ACTH in human urine.

METHODS OF ASSAY

The measurement of adrenal ascorbic-acid depletion in hypophysectomized rats as devised by Sayers, Sayers and Woodbury (3) is undisputedly the most sensitive method for measuring corticotropin. It has been used by the majority of investigators in the field. The adrenal weight-maintenance method (4) has been employed by some workers (5, 6) to corroborate the finding of corticotropin in plasma extracts by the test of Sayers and associates. The adrenal weight-maintenance test is approximately 1,000 times less sensitive than the ascorbic-acid depletion method and is, therefore, unsuitable for work with human blood.

The technic of Sayers and associates has certain limitations which, unless fully appreciated, can lead to erroneous conclusions. The assay rats must be completely hypophysectomized to avoid errors due to endogenous corticotropic activity. Ascorbic-acid concentrations of less than 300 mg. per 100 Gm. in the left or control ("L") adrenal denote incomplete hypophysectomy. In this laboratory, statistical analysis of the control data ("L-R" values)[2] has shown that ascorbic-acid depletions of less than 20 mg. per 100 Gm. of adrenal tissue are insignificant. When the test is used for quantitative purposes, standard doses of corticotropin must be assayed with each unknown. However, satisfactory regressions have not been obtained consistently, and we have come to the conclusion that the quantitative assay of corticotropin in our hands is somewhat capricious. Consequently, our effort has been limited to detection of corticotropin in blood, and we have not attempted to express the results in any but qualitative terms.

The method of Sayers and associates (3) has been modified by Gray and Parrott (7). These investigators add from 0.5 to 1.0 milliunit of standard corticotropin per assay rat either to the plasma to be extracted or to the final solution to be assayed. The "L-R" values obtained are then compared with "L-R" values from another group of rats into which the same quantity of standard corticotropin alone has been injected. If the animals receiving corticotropin plus plasma extract show greater responses than animals receiving the same amount of corticotropin alone, the difference is regarded as being due to the additive effect of corticotropin in the patient's plasma. This procedure of employing "booster" doses of corticotropin is dangerous because of (*a*) the inherent variability of responses and (*b*) the lack of control studies to rule out potentiation of the standard corticotropin by factors in plasma other than endogenous corticotropin. Furthermore, the rationale of this procedure seems questionable. Gray and Parrott have

[2] L-R = difference between concentrations of ascorbic acid in left and right adrenal glands, in mg. per 100 Gm. of gland tissue.

TABLE 1. ADRENOCORTICOTROPIC ACTIVITY IN THE BLOOD OF NORMAL PERSONS

Reference	Cases	Source	Preparation	Ml. eq.* per rat	Adrenocorticotropic activity		
					Present	Range "L-R"†	Corticotropin (milliunits per 100 ml.)
Sayers and associates (8)	2	Plasma	Lyophilization	2	No	9 to 16	10
Bornstein and Trewhella (9)	12	Plasma	Acid-acetone	1.2	Yes	86 to 106	197 ± 39
Upson (10)	8	Serum	Acid-acetone‡	12	No	10 to 36	
	6	Serum	Frozen 1 day	2.5	No	−9 to 10	
Parrott (5)	5	Plasma	Acid-acetone		Yes		59 to 103
Granirer (11)		Plasma			No		
Montanari and associates (12)	8	Plasma	Fresh	0.5	Yes	37 to 77	160
		Plasma	8 hours old	0.5	Yes		80
		Plasma	12 hours old	0.5	Yes		
Rossi and associates (13)	5	Plasma	Fresh		Yes	36	
		Plasma	Ultrafiltration	0.5	Yes	47	
Ceresa and Reyneri (14)	12	Plasma	Acid-acetone	1.2	Yes	81 to 97§	108 to 173
Sydnor and Sayers (15)		Blood	Oxycellulose		No		
Gray and Parrott (7)	6	Plasma	Acid-acetone	0.5 to 1.16	Variable		0 to 91‖
Sydnor and associates (16)	9	Blood	Oxycellulose	20 to 40	No	−25 to 19	
Paris (17)	2	Plasma	Acid-acetone	1.2	No	−11 to 4	
Sydnor and associates (18)	7	Blood	Oxycellulose	25 to 50	No	−21 to 42	

* Ml. eq. = Milliliters equivalent serum, plasma, or whole blood.
† Method of Sayers, Sayers and Woodbury (3). "L-R" = Mean adrenal ascorbic-acid depletion in mg./100 Gm. of adrenal tissue. L = Control adrenal. R = Adrenal removed one hour after injection of test substance. Negative values indicate that "R" adrenal ascorbic-acid concentration is greater than "L." "L-R" values less than 20 are insignificant.
‡ See Table 6.
§ Equivalents to corticotropin standard calculated on basis of "L-R" per 100 Gm. of rat weight instead of adrenal weight.
‖ Booster dose of standard corticotropin added to plasma to be extracted or to final solution to be tested.

stated that the "booster" dose of corticotropin raises the response above the less reliable level usually obtained with doses of 0.25 to 0.50 milliunit of corticotropin. Their data, however, show the corticotropin level of plasma to be from 0.6 to 30 milliunits per milliliter. These high values should be readily measurable without recourse to fortification of plasma with standard corticotropin.

PREPARATION OF BLOOD

The simplest procedure is to inject plasma or serum into the assay rats shortly after the blood sample has been obtained. Employing plasma or serum freshly obtained, or after refrigeration for no longer than twenty-four hours, we have demonstrated corticotropic activity in the blood of patients with a variety of conditions but not in the blood of normal persons

TABLE 2. ADRENOCORTICOTROPIC ACTIVITY OF HUMAN BLOOD IN PREGNANCY, THE PUERPERIUM, AND COMPLICATIONS OF PREGNANCY

Reference	Cases	Source	Preparation	Ml. eq. per rat	Adrenocorticotropic activity		
					Present	Range "L-R"	Corticotropin (milliunits per 100 ml.)
Upson (10)	6	Serum, normal pregnancy	Frozen 1 day	2.5	Variable	8 to 37	
	1	Serum, threatened abortion, during steroid therapy	Frozen 1 day	2.5	No	7	
	2	Serum, pre-eclampsia	Frozen 1 day	2.5	Variable	5 to 37	
Granirer (11)		Plasma, postpartum	Pooled 1 day	0.5 to 2	Yes	17 to 158	
		Plasma, postpartum	Pooled, 5 wks. at 4° C.	0.5 to 2	Yes	17 to 57	
		Plasma, postpartum	Pooled, 8 wks. at 4° C.	0.5 to 2	No	0	
Parrott (5)	1	Plasma, pregnant diabetic	Acid-acetone		Yes		194
Gray and Parrott (7)	8	Plasma, pregnant diabetic	Acid-acetone	0.35 to 0.70	Variable		0 to 310*
	1	Plasma, normal pregnancy	Acid-acetone	0.84	Yes		69*
Paris (17)	7	Serum, normal pregnancy	Fresh	2	No	−18 to 20	
	3	Plasma, normal pregnancy†	Fresh	2	No	4 to 15	
	2	Plasma, postpartum, lactating	Fresh	2	No	3 to 5	
	1	Plasma, postpartum	Fresh	2	No	−13	

* Booster dose of standard corticotropin added to plasma to be extracted or to final solution to be assayed.
† Plasma injected into assay rats within 75 min. (Case 1), 20 min. (Case 2), and 10 min. (Case 3) of withdrawal of blood from patients.

(Tables 1, 2, 3, 4 and 5). These negative results with normal blood indicate that corticotropin, if present, had a concentration of less than 0.1 milliunit per milliliter.

Since the intravenous administration of more than 2.5 ml. of plasma is lethal to rats, Upson (10) attempted to process several milliliters of normal blood into a small volume of a nontoxic preparation containing the corticotropic activity. Table 6 shows such preparations to be devoid of activity. However, Bornstein and Trewhella (9), using a modification of the acid-acetone method of Lyons for the extraction of mammotropin (20), reported high corticotropic activity in the plasma of normal persons and of patients with various disorders. Similar findings were also reported by Parrott (5), Ceresa and Reyneri (14, 19), and Gray and Parrott (7). The acid-acetone method effected no appreciable concentration of plasma corticotropin, since the amount of extract given per rat did not exceed 1.2 ml.-equivalent of plasma. Bornstein, Gray and Parrott (6) attributed the success of this method to the prevention of inactivation of corticotropin

TABLE 3. ADRENOCORTICOTROPIC ACTIVITY IN BLOOD IN HYPERFUNCTIONING ADRENOCORTICAL STATES

Reference	Cases	Source	Preparation	Ml. eq. per rat	Adrenocorticotropic activity		
					Present	Range "L-R"	Corticotropin (milliunits per 100 ml.)
Bornstein and Trewhella (9)	3	Plasma, Cushing's syndrome	Acid-acetone	1.2	Yes	138	440–560
Upson (10)	2	Serum, Cushing's syndrome	Frozen 1 day	2.5	No	−6 to 6	
	1	Serum, Cushing's syndrome and acromegaly	Frozen 1 day	2.5	Yes	60	
	2	Serum, adrenogenital syndrome, untreated	Frozen 1 day	2.5	Variable	4 to 44	
Parrott (5)	1	Plasma, Cushing's syndrome	Acid-acetone		Yes		212
	2	Plasma, Cushing's syndrome, x-ray therapy	Acid-acetone		Yes		132 to 134
Gray and Parrott (7)	4	Plasma, Cushing's syndrome	Acid-acetone	0.5	Yes		91 to 305*
Sydnor and associates (16)	5	Blood, adrenogenital syndrome	Oxycellulose	20 to 40	Yes	29 to 62	
	7	Adrenogenital syndrome during cortisone therapy	Oxycellulose	20 to 40	No	−18 to 19	
	6	Adrenogenital syndrome after cortisone therapy	Oxycellulose	20 to 40	Variable	7 to 57	
Paris (17)	1	Serum, Cushing's syndrome	Fresh	3	No	−8	
	1	Serum, adrenogenital syndrome	Fresh	2	No	−12	
Sydnor and associates (18)	2	Blood, Cushing's syndrome	Oxycellulose	25	No	−41 to 22	

* Booster doses of standard corticotropin added to plasma to be extracted or to final solution to be assayed.

by blood.[3] The factor of time elapsed between withdrawal of blood from the donor and its processing was considered to be crucial. Any delay in processing (for instance, the delay imposed by separating serum) was supposed to favor enzymatic destruction of corticotropin.

Contrary to this view are the following considerations: First, the rate of inactivation of corticotropin in homologous blood is not known.[4] Second,

[3] In support of the concept that blood may inactivate corticotropin, Bornstein, Gray and Parrott (6) cited the work of Pincus and associates (21) on enzymatic inactivation of corticotropin by blood. However, the work of Pincus and associates (21), and also that of Reiss and associates (22), show only the *in vitro* inactivation of corticotropin by plasma, blood and various media of species other than the source of corticotropin. Geschwind and Li (23), on the other hand, found little inactivation of sheep corticotropin by rat plasma, and Richards and Sayers (24) found rat corticotropin to be stable for at least one hour in rat blood *in vitro* at body temperature. In this laboratory (17), plasma of adrenalectomized rats tested for corticotropin thirty minutes after withdrawal of blood was found to possess pronounced activity. It thus appears that there is little evidence that inactivation of corticotropin by homologous blood is an important factor.

[4] Montanari and associates (12) reported 49 per cent inactivation of human endogenous corticotropin by allowing plasma to stand for four hours at 18° C. After twelve

TABLE 4. ADRENOCORTICOTROPIC ACTIVITY OF BLOOD IN HYPOFUNCTIONING ADRENOCORTICAL STATES

Reference	Cases	Fraction assayed	Preparation	Ml. eq. per rat	Adrenocorticotropic activity		
					Present	Range "L-R"	Corticotropin (milliunits per 100 ml.)
Bornstein and Trewhella (9)	1	Plasma, Simmonds' disease	Acid-acetone	1.2	Yes	28	28
	1	Plasma, pituitary dwarfism and obesity	Acid-acetone	1.2	Yes		112
Upson (10)	1	Serum, panhypopituitarism, myxedema	Frozen 1 day	2.5	No	9	
	2	Serum, Addison's disease	Frozen 1 day	2.5	Yes	38 to 48	
	1	Addison's (above) treated with desoxycorticosterone acetate	Frozen 1 day	2.5	No	2	
Parrott (5)	1	Plasma, Simmonds' disease	Acid-acetone		No		
Sydnor and Sayers (15)	3	Blood, Addison's disease	Oxycellulose		Yes		2 to 4
Sydnor and associates (18)	3	Blood, Addison's disease before therapy	Oxycellulose	25	Yes	60 to 215	
	2	Blood, Addison's disease during therapy	Oxycellulose	25	Yes	73 to 131	

the fact that endogenous corticotropic activity has been demonstrated in plasma and serum stored for twenty hours (2, 10, 17) suggests that the inactivation is neither rapid nor complete. In any event, if the high concentrations reported by Bornstein and Trewhella (9), Parrott (5) and Ceresa and Reyneri (14) in normal plasma are correct, it should be possible to detect such activity by the injection of plasma into assay rats as rapidly as possible after withdrawal of blood.

The only reports claiming the presence of corticotropic activity in untreated normal plasma are those of Montanari and associates (12) and Rossi and associates (13). In this laboratory (Table 2) the plasma of women similarly studied failed to show corticotropic activity. The possibility remains that the acid-acetone method actually liberates corticotropin bound to plasma or removes inhibiting substances. This possibility can be tested only empirically, and it can only be pointed out that Sydnor and Sayers (15), Richards and Sayers (25) and Paris (17) were unable to confirm the results obtained with the acid-acetone procedure.

Sydnor and associates (15, 16, 18, 26) processed blood by the oxycellulose method of Astwood and associates (27, 28) for the extraction of pituitary corticotropin. Injecting concentrates equivalent to 20 to 50 ml. of whole

hours the activity had almost entirely disappeared. However, since no other group of investigators has found corticotropin in the unprocessed plasma of normal persons (2, 8, 10, 11, 17), the foregoing data on inactivation cannot be accepted at present without reservation.

TABLE 5. ADRENOCORTICOTROPIC ACTIVITY OF HUMAN BLOOD IN MISCELLANEOUS CONDITIONS

Reference	Cases	Source	Preparation	Ml. eq. per rat	Adrenocorticotropic activity		
					Present	Range "L-R"	Corticotropin (milliunits per 100 ml.)
Bornstein and Trewhella (9)	2	Plasma, hirsutes, no endocrinopathy	Acid-acetone	1.2	Yes		267 to 271
	6	Plasma, cardiac failure	Acid-acetone	1.2	Yes	120 to 129	412±41*
	5	Plasma, above, after therapy	Acid-acetone	1.2	Yes	82 to 106	192±25*
	3	Plasm, "after operations"	Acid-acetone	1.2	Yes	117 to 120	337±35*
	6	Plasma, uncontrolled diabetes without ketosis	Acid-acetone	1.2	Yes	86 to 105	202±27*
Upson (10)	6	Serum, myxedema	Frozen 1 day	2.5	Variable	3 to 52	
	3	Serum, exophthalmic goiter	Frozen 1 day	2.5	Variable	4 to 29	
	1	Serum, acromegaly	Frozen 1 day	2.0	Yes	48	
	1	Serum, acromegaly, hypertension and congestive heart failure	Frozen 1 day	2.5	Yes	34	
		Serum, same case after hypophysectomy	Frozen 1 day	2.0	No	6	
	1	Serum, severe dysmenorrhea	Frozen 1 day	2.5	Yes	34	
		Serum, same case without dysmenorrhea	Frozen 1 day	2.5	No	−9	
Parrott (5)	1	Plasma, diabetes	Acid-acetone		Yes		55
	1	Plasma, "adipose gynism"	Acid-acetone		Yes		214
Ceresa and Reyneri (19)	10	Plasma, rheumatoid arthritis	Acid-acetone		Variable	−19 to 45	0 to 49
Gray and Parrott (7)	4	Plasma, "adipose gynism"	Acid-acetone	0.6	Variable		0 to 212†
	1	Plasma, acromegaly with hirsutism	Acid-acetone		Yes		243†
Sydnor and associates (16)	1	Blood, acute glomerulonephritis	Oxycellulose	40	No	14	
	1	Blood, "idiopathic hypoglycemosis"	Oxycellulose	40	Yes	69	
	2	Blood, acute rheumatic fever with carditis	Oxycellulose	40	Yes	126 to 145	
		Blood, 1 of above during cortisone therapy	Oxycellulose	40	No	−19	
	1	Blood, acute rheumatic fever without carditis	Oxycellulose	40	No	17	
	2	Blood, Sydenham's chorea	Oxycellulose	40	Variable	−8 to 13	
Sydnor and associates (18)	2	Blood, miliary tuberculosis	Oxycellulose	25	No	−4 to −18	
	1	Blood, anorexia nervosa	Oxycellulose	40	No	−2	
Paris (17)	4	Plasma, hypertension 0.5 hour after 50 micrograms histamine	Frozen 1 day	2.5	No	−34 to 13	
	1	Plasma, pheochromocytoma, sustained hypertension	Frozen 1 day	2.0	Yes	36	
		Plasma, same 2 days after removal of pheochromocytoma	Frozen 1 day	2	No	15	
		Same 10 days after removal of tumor	Frozen 1 day	2	Yes	39	
		Same 15 days after removal of tumor	Frozen 1 day	2	No	−9	
	1	Plasma, pheochromocytoma, paroxysmal hypertension					
		a) Before attack, blood pressure normal	Frozen 1 day	2	No	−4	
		b) Twenty minutes after histamine-induced attack	Frozen 1 day	2	Yes	25	
		c) Nine days after removal of tumor and 20 minutes after negative reaction to histamine test.	Frozen 1 day	2	No	6	

* Standard deviation.
† Booster doses of standard corticotropin added to plasma to be extracted or to final solution to be assayed.

TABLE 6. ASSAY OF VARIOUS FRACTIONS OF HUMAN SERUM FOR ADRENOCORTICOTROPIC ACTIVITY

No.	Patient	Serum	Preparation of serum	Ml. eq. per rat	Adrenocorticotropic activity "L-R"
1	Normal	Fresh	Serum 1+acid-acetone 4. Neutralize supernatant. Add acetone 20. Test ppt.	10	12
2	Normal	Fresh	Serum 1+acetone 1. Discard ppt. Add acetone qs. ad. 85%. Test ppt.	10	−4
3	Normal	Frozen 2 weeks	Serum 1+saline 1+acetone 2. Discard ppt. Acetone qs. ad. 95%. Test ppt.	8	8
			Test supernatant after evaporation.	10	−10
4	Normal	Fresh	Serum 1+saline 1+acetone 2. Discard ppt. Acetone qs. ad. 95%. Test ppt.	10	37
5	Normal	Fresh	Serum 1+ethanol 1. Discard ppt. Ethanol qs. ad. 85%. test ppt.	10	5
6	Normal	Fresh	Serum+ethanol. qs. ad. 65%. Discard ppt. qs. ad. 95%. Test ppt.	10	2
			As above, test supernatant after evaporation.	15	10
7	Normal	Fresh	Serum 1+2% H_2SO_4 1 at 100° C. for 15 minutes. Add 68% trichloroacetic acid qs. no further ppt. Wash and Test ppt.	10	17
8	Normal	Fresh	Serum 1+butanol 6. Discard ppt. Evaporate supernatant, dissolve in water 20, add 1% phosphotungstic acid. Test ppt.	10	−19
9	Addison's disease	Fresh	Same as 1.	10	5
10	Addison's disease	Fresh	Same as 4, but supernatant tested after evaporation.	10	−9
11	Acute leukemia during corticotropin therapy	Fresh	Same as 4. Ppt. tested.	10	−8
12	Ulcerative colitis during corticotropin therapy	Fresh	Same as 4. Ppt. tested.	11	6
			Supernatant tested.	14	−17

blood into each assay rat, these investigators found corticotropin in Addison's disease, the adrenogenital syndrome and a few miscellaneous conditions (Tables 3, 4 and 5). No activity was detected in normal persons, in patients with Cushing's syndrome, or in febrile patients with miliary tuberculosis (Tables 1, 3, and 5). These results are in general confirmatory of those reported by Taylor and associates (2) and indicate further that the concentration of corticotropin in normal blood must be less than 0.005 milliunit per milliliter. Injections of extracts obtained by the oxycellulose method appear to be superior to injections of 2 ml. of untreated serum, since all the cases of adrenogenital syndrome reported by Sydnor and associates (16) had demonstrable corticotropic activity, whereas only a few of our cases with this syndrome showed such activity. On the other hand, the oxycellulose extracts appear to be no better than untreated serum in conditions in which it is generally conceded that corticotropin is present (Addison's disease), or in which corticotropin is not generally found (normal). From the clinical point of view, the oxycellulose extraction of corticotropin does not appear practical, since it requires a minimum of 20 to 50 ml. of blood per assay rat.

SURVEY OF CORTICOTROPIN IN HUMAN BLOOD

Corticotropic activity in normal human blood (Table 1) has been found by three groups of investigators (5, 7, 9, 12–14), but not by others (8, 10, 11, 15, 17, 18, 25). In the second or third trimester of pregnancy corticotropic activity has been found occasionally (Table 2). Postpartum plasma has not been uniformly found to contain detectable amounts of corticotropin. The high levels found in diabetic pregnant women (6, 7) must await confirmation.

In hyperfunctioning adrenocortical states, corticotropin has been found in the adrenogenital syndrome, but not uniformly in Cushing's syndrome (Table 3). In hypofunctioning adrenocortical states, corticotropin has been found consistently in untreated Addison's disease (Table 4). Bornstein and Trewhella (9) reported high levels of corticotropin in a case of Simmonds' disease. This finding was not confirmed by others (4, 7).

The corticotropic activity of blood in miscellaneous conditions is shown in Table 5. The titers obtained by the acid-acetone process seem extraordinarily high. Work with untreated plasma and serum (7,17), and with oxycellulose extracts of whole blood (15, 16, 18) indicates that myxedema, acromegaly, pheochromocytoma and some acute stressful conditions may be associated with demonstrable titers of corticotropin in blood.[5]

SUMMARY

The various procedures employed in the assay for corticotropin in human blood have been described and analyzed. Only two of these procedures—the application of the assay method of Sayers and associates to untreated serum or plasma and to extracts prepared by the oxycellulose process—have to the present time yielded results which seem to be compatible with one another. The results obtained with these two procedures indicate that corticotropin is measurably present in the blood of patients with untreated Addison's disease, adrenogenital syndrome, and a few miscellaneous disorders. Corticotropin is not demonstrable in the blood of patients with Cushing's disease, nor in the blood of normal persons.

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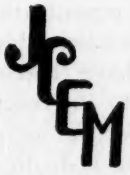

CORTICAL AND MEDULLARY ADRENAL ACTIVITY IN SURGICAL AND ALLIED CONDITIONS

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AN INCREASED activity of the pituitary-adrenocortical system has been repeatedly demonstrated as part of the reaction pattern of the organism to stressful situations. The response of the adrenal medulla and the adrenergic nervous system to stress is, however, less clearly defined. The adrenal medulla is brought into increased activity by a variety of emotional stimuli, as demonstrated experimentally by Cannon and his associates (1), but the evidence for medullary hypersecretion in other situations of stress is scanty. In heavy muscular work causing fatigue, a hypersecretion of medullary hormones has been demonstrated in unanesthetized dogs (2) and in man (3). Afferent stimulation of the sciatic nerve or the brachial plexus in the anesthetized cat elicits an increased secretion of medullary hormones, especially adrenaline (4).

Surgical operations and injuries no doubt represent stress-provoking factors. Clinical and experimental investigations have indicated that the pituitary-adrenocortical system is involved in the reactions of the organism evoked by surgery (5–9). We have therefore considered it of interest to obtain further information as to the reaction of the adrenal cortex and medulla in such cases. Adrenocortical activity has been estimated by determination of the blood level of 17-hydroxycorticosteroids (17-OH), and the adrenal medullary activity by the rate of adrenaline excretion in urine. The activity of the adrenergic nervous system has been estimated by determining the noradrenaline excretion in urine.

The adrenaline output in urine may be regarded as a relative measure of adrenal medullary activity, since it has been demonstrated (10) that after adrenalectomy most of the adrenaline disappears from the urine, whereas the excretion of noradrenaline, which is chiefly derived from the adrenergic nerves, remains at the previous level.

In addition, the eosinophil count in the blood has been used as an indicator of a reaction of the organism to stress. The mechanism governing

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the blood eosinophils is complex and involves both the cortical and the medullary systems (11).

One of the main difficulties in evaluating the activity of the adrenal cortex in clinical cases has been the lack of reliable methods measuring the changes in this activity. Methods have been developed whereby cortical activity may be investigated indirectly by the measurement of adrenocortical steroids in the urine (17-ketosteroids, 11-oxysteroids), as well as by their effect on blood cells (eosinophils, neutrophils, lymphocytes, and on protein (creatinine), carbohydrate (blood sugar) and electrolyte metabolism. Eosinophil counts and measurement of 17-ketosteroid excretion seem to have been the methods most commonly used.

The steroids elaborated by the adrenal may be classified into three major groups: 1) desoxycorticosterone-like compounds, which mainly affect water and electrolyte metabolism; 2) compounds like 17-hydroxycorticosterone (compounds E and F) which mainly affect carbohydrate and protein metabolism; and 3) compounds with the properties of the sex hormones.

At the present time there is no specific method available for determining the secretion of the compounds in the first group. Nelson and Samuels (12) have recently developed a method of determining the 17-hydroxycorticosteroids in the blood. Some of the sex hormones are excreted as 17-ketosteroids.

MATERIAL AND METHODS

Twenty-seven patients in the surgical department of the Karolinska Hospital, who were operated upon or had suffered traumatic injury were followed with daily determinations in blood and urine. The operations were major—cholecystectomies or more extensive procedures. All endocrine cases were excluded from this study. Generally blood samples were withdrawn at 8 A.M. and at the end of the operation.

The level of 17-OH in the blood was determined according to the method described by Nelson and Samuels (12). Approximately 15 ml. of heparinized plasma was extracted with chloroform. Chromatography was carried out on magnesium silicate-Celite columns. The sample was dissolved in 0.2 ml. of methanol, 0.3 ml. of the phenylhydrazine-sulphuric acid solution was added, and the whole was incubated at 60° C. for one hour. After incubation the samples were read in 0.5-ml. microcuvettes on the Beckman spectrophotometer at 370, 410 and 450 mμ. Correction for background absorption was made by using the absorption factor given by Nelson and Samuels (12). The concentration of 17-OH is expressed in μg. per 100 ml. of plasma. The normal concentration is 4–10 μg. (Table 1).

The eosinophils were counted according to a method described by Rud (13).

Adrenaline and noradrenaline in urine were prepared and estimated according to the methods of Euler and Hellner (14) and Euler (15). The methods consist in principle of adsorption of the urinary catechol amines on aluminum hydroxide and biologic assay on the cat's blood pressure and on the fowl's rectal cecum. From the assay results, the concentrations of adrenaline and noradrenaline can be calculated separately.

TABLE 1. BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS BEFORE AND AFTER OPERATION, IN PATIENTS WITH "NORMAL" SURGICAL COURSE

No. of patients	Conc. of 17-OH in plasma		Duration of increased conc. of 17-OH in blood postop. (hrs.)
	Before operation (μg./100 ml.)	After operation (μg./100 ml.)	
11	5.6± 0.9* (2.1–13.0)†	31.4± 1.9 (20.6–50.0)	38 (24–72)

* Standard error of the mean.
†Range.

Urine was collected in 24-hour periods from 8 A.M. to 8 A.M., was kept cold during collection and acidified with HCl to pH 3. Aliquots of 300 ml. of urine from the total 24-hour specimen were used for the catechol estimations.

The normal excretion figures for hospitalized patients in bed, not subjected to stress, may be given as approximately 5 μg. of adrenaline and 20 μg. of noradrenaline per twenty-four hours, with standard deviations of about 2 and 10 μg. respectively.

RESULTS

I. Adrenocortical activity

The effects on adrenocortical activity of operative procedures have been studied before, during and after operation.

A. *Adrenocortical activity before operation*

During the hours before operation the patient is more or less under psychic tension, which may stimulate the adrenal cortex. Roche *et al.* (8) found a gradual progressive lowering of the eosinophil count as the day of operation approached.

On the morning of the day of operation the blood levels of 17-OH were usually elevated in our patients, though not to a significant degree.

Following preoperative preparation (0.01 Gm. of morphine and 0.04 mg. of scopolamine subcutaneously) and spinal anesthesia or Pentothal induction, the blood level of 17-OH was increased in 3 patients. In these instances there was a significant stimulation of the adrenal cortex, which may be a summation effect of tension, preoperative preparation and anesthesia (Table 2).

The blood level of 17-OH was not altered by intravenous transfusions of blood, glucose or saline.

B. *Adrenocortical activity during and after operation*

Immediately after surgery all patients showed a marked elevation of

TABLE 2. CONCENTRATION OF 17-HYDROXYCORTICOSTEROIDS IN THE BLOOD BEFORE, AND IMMEDIATELY AFTER THE OPERATION

Diagnosis	Age of patient (yrs.)	Conc. of 17-OH in plasma (μg./100 ml.)			
		Normal value	½ hour preop.	Duration of operation	1 hour postop.
Chronic salpingitis (salpingectomy)	35	10.5	15.7	50 min.	32.5
Fibromyoma of uterus (myomectomy)	46	1.5– 2.5	16.0	1 hr. 10 min.	25.8
Gastric ulcer (partial gastrectomy)	56	5.1–11.5	23.9 (spin. anesth.)	2 hrs. 20 min.	70.0
Endometrioma of colon (segmental resection)	43	9.2–15.9	27.4 (spin. anesth.)	2 hrs. 45 min.	44.1
Pulmonary tuberculosis (pneumonectomy)	42		24.1 (gen. anesth.)	3 hrs.	33.0

the blood level of 17-OH and a fall in eosinophils, indicative of increased adrenocortical activity (Table 1). During the postoperative period, however, the pattern of adrenocortical activity varied.

Our material has been divided into four groups:

1. Patients with a normal operative and postoperative course (11 cases).
2. Patients in whom shock developed (5 cases).
3. Patients with other complications (4 cases).
4. Patients with traumatic injuries (7 cases).

Group 1. Figure 1 shows 11 cases following a "normal," uncomplicated course. The blood level of 17-OH was increased immediately following operation and remained so for about thirty-eight hours. The level then dropped to subnormal values for twenty-four hours, after which a second elevation, considerably less than the first one, occurred for about forty-eight hours. After that the 17-OH returned to the preoperative level. This mode of reaction seemed to occur independently of the type or the extensiveness of the operation.

Group 2. In each of the 5 cases belonging to this group the shock was severe and fatal. In 3 cases shock developed during the operation; in the other 2 cases, postoperatively (Table 3). Immediately following the operation all patients showed a high level of 17-OH, which remained elevated one to four hours before death.

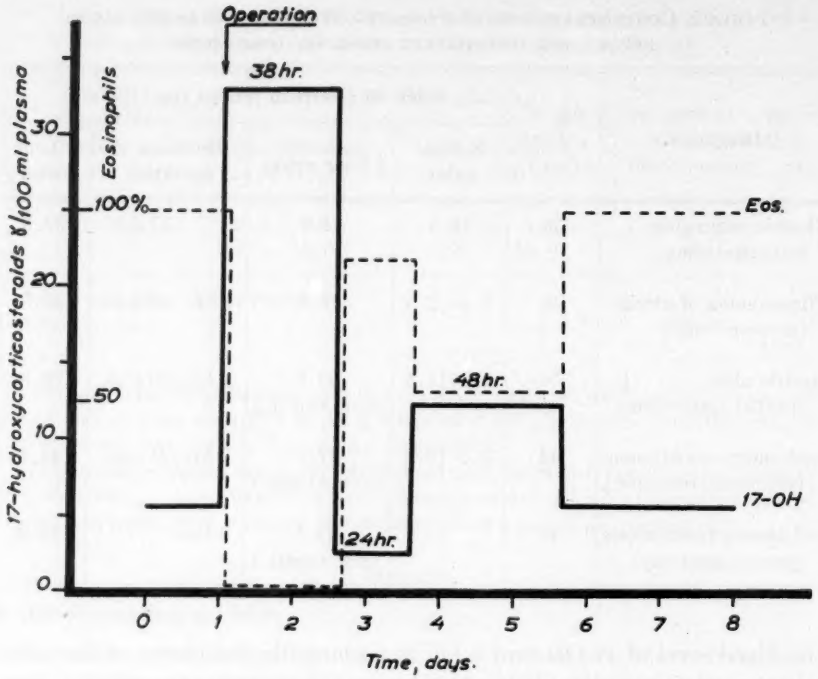

FIG. 1. Mean values of blood levels of 17-hydroxycorticosteroids (17-OH) and eosinophils (Eos) in 11 patients with a normal postoperative course.

Group 3. The 4 patients of this group had less severe complications. Bleeding in 1 case, oliguria in 2 and fever of unknown origin in 1 case, were accompanied by an elevation of the 17-OH concentration.

Group 4. Seven cases belong to this group. In 5 of these the pattern was the same as that in the cases with a normal course described in group 1 (Fig. 2).

Two patients with shock, however, had a low blood level of 17-OH immediately after the trauma. Next day, when the shock had subsided, the 17-OH had reached a high level and then varied as in a normal case following surgery.

II. Adrenomedullary activity

The catechol amine output in urine was followed in a total of 18 patients. Determinations of 17-OH in blood were made in 3 of 10 patients with a normal operative and postoperative course, in 3 patients with operative complications, and in 4 accident cases.

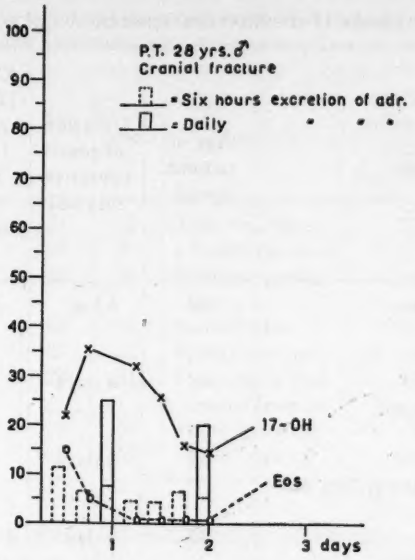

FIG. 2. Eosinophils (Eos), blood levels of 17-hydroxycorticosteroids (17-OH), and adrenaline excretion in a 28-year-old man (P.T.) following cerebral concussion and cranial fracture.

The excretion pattern of adrenaline and noradrenaline showed wide variations in our material. In general, however, the postoperative adrenaline excretion in urine in patients whose recovery was rapid and uneventful differed little from that in hospitalized patients with unstressful ailments. On the other hand urinary adrenaline excretion was increased in patients with complications and in accident cases.

Group 1. Patients with a normal operative and postoperative course. In 40 determinations from 10 patients with uneventful recovery, covering a period from one day before operation through seven days after operation, the adrenaline excretion was 4.2 (S.D. = 2.2) μg. per twenty-four hours. In these uncomplicated cases the noradrenaline excretion was less regular. Although it was within the normal range for 6 of the 10 patients, noradrenaline excretion was definitely increased in the remaining 4, with values between 82–132 μg. per twenty-four hours, on the third to the seventh day after the operation. High noradrenaline excretion is, however, a more or less constant feature in groups 3 and 4 (following), in which a considerable degree of stress was characteristic of the condition.

The average daily adrenaline and noradrenaline excretion values in the

TABLE 3. BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS IN POSTOPERATIVE SHOCK

Diagnosis	Age of patient (yrs.)	Duration of post-operative survival	Conc. of 17-OH in plasma, 1–4 hrs. before death (μg/100 ml.)	Adrenals at autopsy
Pulmonary tuberculosis (cardiac arrest)	42	5 hrs.	33.0	Normal
Ca. body of uterus (primary hemorrhage)	66	3 days	33.4	Normal
Chronic cholecystitis (lig. of portal vein and rt. hep. art.)	67	2 days	27.6	Normal
Gastro-colic fistula (leaking anastomosis)	47	1 day	67.0	Normal
Ca. cardiac end of stomach (leaking anastomosis)	66	3 days	40.9	Normal

10 patients with uneventful recovery for one day before and seven days after the operation are given in Table 4.

In 2 cases (B.S. and I.E., Table 5), however, following clinically uncomplicated operations, an increased catechol amine secretion was noted.

TABLE 4. URINARY ADRENALINE AND NORADRENALINE (μG. PER 24 HRS.) IN 10 UNCOMPLICATED SURGICAL CASES

Days before or after op.		1	Operation	1	2	3	4	5	6	7
Conc. in urine (range)	Adrenaline Noradren.	1.4–4.1 5–41	1.3–7.1 6–37	1.0–8.2 15–28	2.8–6.0 9–90	3.1; 4.3 28; 132	3.4 82	1.4–10 8–45	3.1–8.5 10–99	1.3–6.7 6–58
No. of dets.		5	6	6	3	2	1	5	4	5
Average conc.	*Adrenaline* *Noradren.*	*3.0* *15*	*3.2* *17*	*4.7* *23*	*3.8* *46*	*3.7* *80*	*3.4* *82*	*4.1* *26*	*5.8* *48*	*4.1* *25*

The adrenaline and noradrenaline values found in group 1 agreed fairly well with those observed in a group of 7 hospitalized patients not subjected to surgery or major stress. These patients excreted 4.5 μg. of adrenaline (S.D. = 1.8) and 44 μg. of noradrenaline (S.D. = 25) per twenty-four hours.

Although not too much weight can be attached to the single values or

TABLE 5. MAXIMAL CATECHOL AMINE EXCRETION IN 2 CASES WITHOUT CLINICAL COMPLICATIONS AND IN 6 CASES WITH COMPLICATIONS OR ACCIDENTS

Group No.	Subject	Age (yrs.)	Sex	Diagnosis	Highest adrenaline output (μg./24 hrs.)	Highest noradrenaline output (μg./24 hrs.)
1	B.S.	21	M	Duodenal ulcer	25	114
1	I.E.	32	F	Cholecystectomy	65	74
3	V.L.	55	M	Bleeding gastric ulcer	64	153
3	S.M.	48	M	Gastric ulcer	30	168
4 (3)	L.M.	37	M	Spinal fracture	54	94
4	P.T.	28	M	Concussion and cranial fracture	57	84
4	L.G.	50	M	Pelvic fracture	12	125
4	B.K.	36	M	Facial burn	39	442

averages on account of the limited number of estimations, it is nevertheless clear that, although there was no noteworthy increase in adrenaline excretion after surgical intervention in this group, there was a definite increase in the noradrenaline excretion in several cases.

Data on 1 case are presented in Figure 3, including values for 17-OH blood levels.

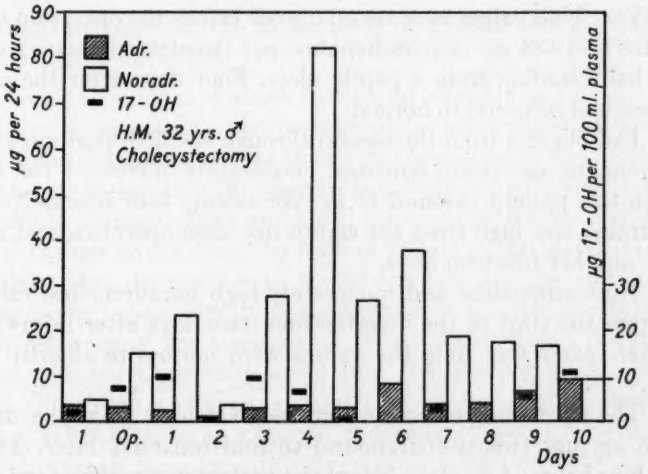

FIGURE 3

Group 2. Patients in whom shock developed. These patients were not investigated for catechol amines in the urine.

Group 3. Patients with other complications. In Patient V.L. with a bleeding peptic ulcer, the catechol amine excretion was very high. One patient (L.M.) excreted a large amount of adrenaline at the time of an attack of fever of unknown origin. A high adrenaline excretion appeared during a period of oliguria in Patient S.M.

Group 4. Patients with traumatic injuries. The 4 accident cases were: 1 fractured skull, 1 fractured spine, 1 fractured pelvis and 1 moderately severe facial burn (3 per cent). All patients recovered from their injury.

The catechol excretion was well above normal during some period after the trauma or operation in all of the 6 patients belonging to groups 3 and 4. Because of the large variations it is not possible to present a typical course or average values, but an idea of the changes is given in Table 5 where the maximal excretion figures are tabulated.

Comments on the subjects in Table 5

B.S. There was a moderately high adrenaline excretion two days after the operation (25 μg. per twenty-four hours) but it soon returned to normal. The noradrenaline excretion was slighly elevated on several occasions for three days before and four days after surgery.

I.E. Two days after surgery the adrenaline values were very high (65 and 49 μg. per twenty-four hours) and excretion of noradrenaline was moderately elevated. Five days after surgery catechol excretion was normal.

V.L. Very high values were noted a week before the operation (64 μg. of adrenaline and 133 μg. of noradrenaline per twenty-four hours) when the patient had bleeding from a peptic ulcer. Four days after the operation the values had returned to normal.

S.M. had oliguria from the second through the fifth postoperative day. The adrenaline excretion remained moderately increased for almost a month in this patient (around 15 μg. per twenty-four hours) Noradrenaline excretion was high from the eighth day after operation and remained high for another fourteen days.

L.M. High adrenaline and moderately high noradrenaline values were found from the start of the investigation (two days after injury) to nine days later. After this time the values were normal or slightly elevated (Fig. 5).

P.T. The adrenaline excretion remained elevated from the day of injury (25 μg. per twenty-four hours) to fourteen days later (19 μg. per twenty-four hours), four days before the patient was ambulatory. The noradrenaline excretion was within the normal range except for 1 high value (84 μg. per twenty-four hours), four days after the trauma (Fig. 2).

L.G. This patient suffered a fractured pelvis sixteen days before the first catechol amine estimations were made. The adrenaline excretion was low during the following month in the hospital; the noradrenaline excretion was high (up to 125 μg. per twenty-four hours) on several occasions.

B.K. A different excretion pattern was observed, with an extremely high noradrenaline output on the day after the trauma (442 μg. per twenty-four hours) and an initial high adrenaline excretion, which in the following week was consistently low (Fig. 4).

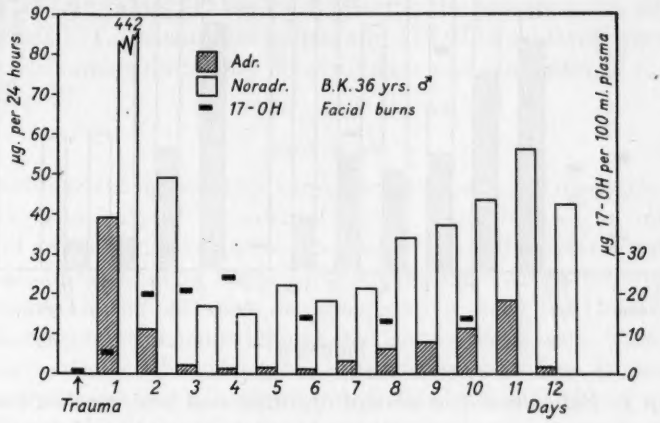

FIGURE 4

III. Relationship between the blood level of 17-OH and the adrenaline and noradrenaline excretion

It has been the subject of much discussion whether an increased pituitary-adrenocortical activity can be elicited by adrenaline. Although this undoubtedly is the case in rats, as shown by the extensive and careful work of Long and his associates (16), there is accumulating evidence that this is not the case in humans. Thus prolonged infusions with even high doses of adrenaline in man do not raise the level of 17-OH in the blood (17). From this it follows that the increase in 17-OH level as observed in the present study may occur independently of an increase in adrenaline secretion.

On the other hand the eosinopenia often demonstrated during stress conditions does not necessarily mean that the adrenocortical activity is increased, since such a change, although to a lesser degree, ensues after adrenaline. What may be an example of this is offered by Patient L.M. during the seventh through ninth days after operation (Fig. 5) when a marked eosinopenia occurred together with low blood 17-OH values and a strongly increased adrenaline excretion.

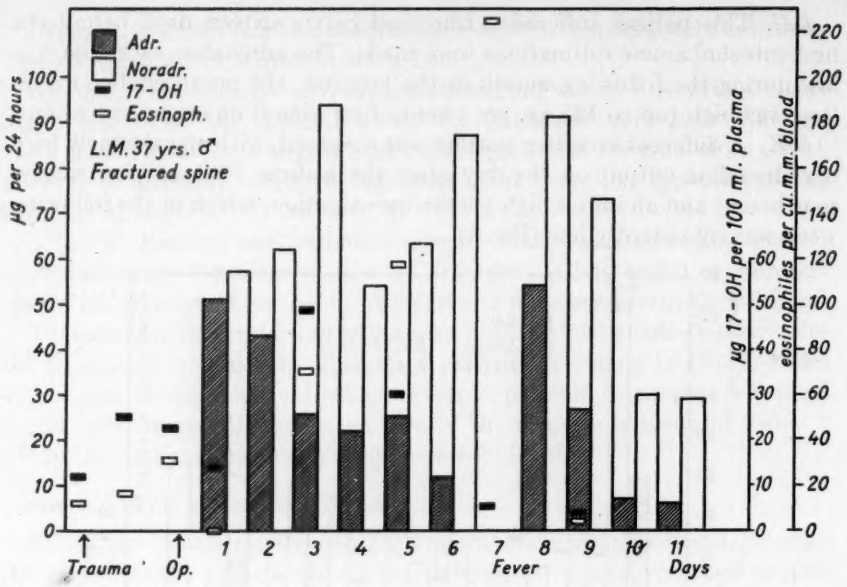

FIGURE 5

Group 1. Patients with a normal operative and postoperative course. All patients showed an increase in the blood level of 17-OH lasting about thirty-eight hours after surgery. Most of them did not show any increase in the excretion of catechols. However, in 2 cases the catechol amine excretion was significantly increased (Table 5). Clinically these patients did not differ from the others. The increase in the noradrenaline secretion observed in some of these patients postoperatively was not accompanied by an increase in the 17-OH level of the blood. This result suggests that a moderately increased activity of the adrenergic nervous system is not, per se, accompanied by an increase in the cortical activity.

Group 2. Patients in whom shock developed. In these patients only the concentration of blood 17-OH was investigated, with which we have dealt previously.

Group 3. Patients with other complications. Although no parallelism was encountered between the catechol amine excretion and the 17-OH blood level, a rise in both was found in all 4 patients. A general similarity in the course of changes occurring in both parameters was noted in Patient V.L. (bleeding), L.M. (fever) and S.M. (oliguria). Particularly striking was the extremely high value for the catechols and 17-OH in Patient V.L. during the preoperative period; both values became normal at the same time during postoperative recovery.

Group 4. Patients with traumatic injuries. In these cases there was a rather good parallelism between the 17-OH levels in the blood and the urinary excretion of adrenaline.

In Patient B.K. (facial burn) a different pattern was observed. Blood 17-OH concentration was moderately but consistently higher than normal during the week following the trauma, although the catechol amine excretion after two days fell to rather low levels.

Occasionally high adrenaline or noradrenaline values occurred together with low 17-OH values (Patient I.E.) during the postoperative period.

In Patient P.T. the catechol output and 17-OH blood levels were measured every six hours during the first two days and both showed increased values (Fig. 2).

DISCUSSION

The results of the present study indicate that a surgical operation causes an increased activity of the adrenal cortex which releases an increased amount of adrenocortical steroids. The blood level of these steroids remains high for about thirty-eight hours, after which time it drops in a characteristic manner. During operative or postoperative shock the blood level of 17-OH increases and remains high as long as the shock lasts. This is true even prior to death, when shock has lasted for several days. Immediately following traumatic shock, however, the 17-OH level in the blood seems to be low. The reason for the absence of reaction in these cases is not clear. It is possible that the shock causes immediate circulatory disturbances in the pituitary gland, inhibiting the production or secretion of ACTH.

The increase in the concentration of 17-OH in the blood following a normal operative procedure can perhaps be used for evaluating the functioning capacity of the patient's pituitary-adrenocortical system. In cases in which the postoperative increase of 17-OH fails to materialize, an insufficiency of this system would be suggested. Substitution therapy with cortisone or ACTH may be appropriate. Secondly, if the level of 17-OH remains high for three days or more after the operation, a postoperative complication might be suspected.

The results of the catechol amine excretion studies in the present group of surgical and traumatic cases clearly demonstrates the participation of either the adrenaline-producing or the noradrenaline-producing systems, or both, in the reaction pattern of the organism in situations which provoke stress. It seems to be the degree of stress, however, more than the magnitude of the operation or injury that determines the intensity of the reactions. On the whole it appears that adrenaline bears a closer correlation to the degree of stress, than does noradrenaline. The mechanism for the activation of the systems, which eventually leads to a release of adrenomedullary

adrenaline during stress is not clear. In the cases in which pain is an integrative part of the symptoms, the release of adrenaline is in agreement with earlier observations. Nevertheless, the possible contribution of other factors cannot be excluded. The point of attack of the stress-provoking forces may be central as well as peripheral, and mediated through afferent fibers to higher levels of the central nervous system, as shown by the reflex liberation of adrenaline from the cat's suprarenals (1, 4) and by the release of adrenaline after direct electrical stimulation of hypothalamic regions (18, 19). The possibility of an adrenomedullary secretion regulated by chemical receptor areas in the hypothalamus other than in the areas regulating the blood sugar level (20), must also be considered.

Our results show that there is not only an increase in cortical activity during conditions of traumatic and surgical stress but also an increase in adrenomedullary activity and in that of the adrenergic nervous system. It is of interest that the two latter increases may occur together or separately, which is in agreement with the opinion expressed earlier (21). In several cases there was a tendency towards an inverse correlation between the 17-OH and noradrenaline figures (Fig. 4).

If both 17-OH and catechol production are increased during stress and are apparently partly independent of each other, which parameter gives the better expression of the condition of the patient? The answer cannot be given at present, but it seems that measurement of each of these factors may be of prognostic and diagnostic value in indicating the severity and course of a diseased state.

SUMMARY

The blood levels of 17-hydroxycorticosteroids (17-OH) were estimated in 27 surgical cases. All cases showed an elevated level immediately following operation. In those with an uncomplicated postoperative course the blood level showed a typical pattern, with high values during the first thirty-eight hours.

Cases in which there was development of shock or other complications showed very high values of 17-OH.

In most patients with a normal operative and postoperative course the adrenaline excretion in urine was approximately within normal limits during the seven days following operation. The noradrenaline excretion was usually normal, but occasionally high values were observed.

In patients with injuries or surgical operations accompanied by complications, the excretion of adrenaline or noradrenaline, or both, was often greatly increased during the period immediately after the trauma.

The opinion is expressed that determination of both the 17-OH blood level and catechol amine excretion may give valuable information as to the physiologic state of the patient.

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PINEAL LESIONS AND PRECOCIOUS PUBERTY: A REVIEW

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THE syndrome of premature sexual maturation (precocious puberty) in man has occurred in association with many factors, including pituitary, adrenal, gonadal and pineal lesions. The first three are well known to cause sexual precocity, but the relation of tumors of the pineal gland to this condition has never been defined. The nature of the association is not generally understood, because the available data on pineal function are not well known. Since this literature has never been thoroughly surveyed, an inclusive review, to be published later, has been undertaken by the author. Portions of it that concern the association of pineal tumors and precocious puberty are summarized in this paper.

The relationship has been known since 1898, when Heubner described the first case. Since that time at least 475 verified cases of pineal tumor have been reported in detail. Of these, 178 occurred in patients 1 to 16 years of age, 145 of whom were boys. Precocious puberty was observed in 46 of these boys.

Examination of the literature reveals that two different explanations of the relationship between pineal tumors and sexual precocity have been offered. The first of these, formulated half a century ago, was that sexual precocity was due to decreased secretion of a pineal hormone that was presumed to retard gonadal development, under normal conditions, until puberty. The decreased production was believed to be caused by destruction of pineal parenchymal cells by neoplasms. This hypothesis has been viewed with skepticism for some time, chiefly because experimental evidence on pineal function is almost entirely unknown. A second hypothesis, currently accepted by most authorities (1–6), is that a pineal tumor can produce precocious puberty through indirect stimulation of the pituitary gland; that is, the expanding tumor is believed to compress or destroy hypothalamic areas that control anterior pituitary function. The trauma presumedly increases the secretion of gonadotropin, with consequent pre-

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cocity. This theory has gained support on the basis of case reports of sexual precocity associated with intracranial lesions not of pineal origin. Its proponents label the pineal body as a nonfunctioning organ; furthermore, they consider the histologic composition of its tumors of no importance.

A preliminary evaluation of these theories may be made after careful examination of the literature. The normal human pineal gland is composed of two main types of cells—intrinsic parenchymal cells and nonparenchymal cells (chiefly neuroglia) (7). Tumors of this gland may similarly be classified as parenchymal and nonparenchymal neoplasms. The parenchymal tumors are pinealomas, pinealoblastomas, and adenomas; the nonparenchymal lesions include mixed tumors, supporting-tissue tumors, and cystomas. This classification is based on histopathologic diagnoses made at autopsy or operation. Because the reported clinical studies vary greatly, both in thoroughness and in length of observation periods, the rate of growth of either type of tumor cannot be precisely determined. However, the evidence at hand suggests that it is similar in both types and that both types of tumor reach about the same size before operation or autopsy.

Parenchymal and nonparenchymal tumors are equally distributed in children 1 to 16 years of age (Table 1). Data for this group alone are pre-

TABLE 1. DISTRIBUTION OF PINEAL TUMORS IN CHILDREN 1 TO 16 YEARS OF AGE

	Parenchymal	Nonparenchymal	Totals
Males	72	73	145
Females	19	14	33
Totals	91	87	178

sented because all the reported cases of sexual precocity occurred in this age range. Ages at autopsy or operation are used, since there is no way of ascertaining ages at onset. According to the pressure hypothesis, the development of precocious puberty is unrelated to the histologic structure of the pineal tumor; therefore, since both types of tumors are found in equal distribution, the condition should occur with equal frequency in association with both types. Actually, however, the number of nonparenchymal tumors associated with precocity is more than three times as large as the number of parenchymal tumors (Table 2). The probability that this is a chance disproportion is less than 1/10,000, as computed by the chi-square technique (8). Statistics for the male cases alone are equally significant. The theory that sexual precocity associated with pineal lesions is produced merely by pressure on the hypothalamus cannot explain the fact that nonparenchymal tumors were found in more than 75 per cent of these cases of

TABLE 2. DISTRIBUTION OF PINEAL TUMORS IN CASES OF PRECOCIOUS PUBERTY

	Parenchymal	Nonparenchymal	Totals
Males	10	36	46
Females	0	0	0
Totals	10	36	46

precocious puberty, although the general incidence of such tumors is only 49 per cent.

The earlier hypothesis—that the pineal gland is an endocrine organ and its secretion inhibits gonadal development—is consistent with the evidence that precocious puberty is usually associated with nonparenchymal tumors. Since these neoplasms destroy parenchymal tissue, the high incidence of precocity associated with them may be due to decrease or loss of pineal secretion. Although a few cases of precocious puberty have been associated with parenchymal tumors, this may be explained by the possibility that such tumors do not necessarily secrete. Indeed, some reports have described the pinealomas associated with precocity as composed of cells so undifferentiated and necrotic that secretion is highly unlikely.

An interesting and often overlooked corollary to the foregoing findings is the association of hypogenitalism with pineal tumors. Thirty such cases, all in male patients, have been described; in 20 the tumors were parenchymal, and in 10 they were nonparenchymal. Although this association of pinealomas and hypogonadism is striking, it is not statistically significant, since it occurred in so few cases and was infrequently observed in cases of pineal tumor in the age range involved (6 to 35 years). Nevertheless, since a pressure hypothesis is employed to explain such cases also, these associations of nonparenchymal tumors and precocious puberty and of parenchymal tumors and sexual retardation warrant careful examination.

The hypotheses discussed here do not offer any explanation for the fact that neither precocious puberty nor hypogonadism has ever been reported in female patients with pineal tumors. A contributing factor may be the very low incidence of pineal tumors in girls.

A tentative conclusion suggested by the data reviewed is that precocious puberty in patients with pineal tumors is not caused by pressure on the hypothalamus. Pressure cannot be entirely excluded as an etiologic factor, however, since precocity associated with extra-pineal intracranial tumors may perhaps be caused by the compression of appropriate hypothalamic centers. On the other hand, the pressure hypothesis does not explain the

pineal gonadal syndrome, in view of the evidence discussed. Final conclusions cannot be reached at this point, since data of the type presented permit no validation, but only delineation, of hypotheses.

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ELASTIC FIBERS IN THE TUNICA PROPRIA OF NORMAL AND PATHOLOGIC HUMAN TESTES*†

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IN THE publications on the components of human testes, few references have been made to the elastic fibers of the wall of the seminiferous tubule. Sniffen (1) mentions that they appear at puberty and increase markedly in old age. Very little has been written regarding the changes brought about in elastic fibers by inflammation or hormonal diseases of the testes (Howard *et al.*) (2). In this study we have attempted to establish the factors which govern the development and maintenance of elastic fibers of the tunica propria, by means of a comparison of their morphology in normal and pathologic conditions. We have not investigated the changes in the elastic tissue of the vessels, tunica albuginea and septa of the testis. It should be borne in mind that the tunica propria of the adult testis, in its normal condition, is a structure of connective-tissue type in which cells, amorphous substance, reticular, collagenous and elastic fibers may be found.

MATERIAL AND METHODS

The material presented here is based on studies made on 46 normal testes and on 74 pathologic specimens.

Testicular biopsies were performed by the Charny and Meranze technique (3). The tissues were sectioned at 5 to 10 microns. Bouin's fixative was used. Weigert's resorcin fuchsin stain was used for delineating the elastic fibers and Orth's lithium carmine stain (4) for the other testicular components.

Fibers are considered to be elastic when, in addition to their fibrillar structure, they are seen to be homogeneous and take a dark blue stain. We did not apply elastinase enzyme to this material.

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RESULTS

1. Normal testes

Adults. Thirty-one adult patients were studied, whose ages ranged between 17 and 79 years. In 16 subjects less than 50 years of age the elastic fibers were homogeneous, undulating, of variable length, stained dark blue, and with a constant diameter of approximately 1 micron. They were always located within the tunica propria, dissociated from the vessels and septa (Fig. 1).

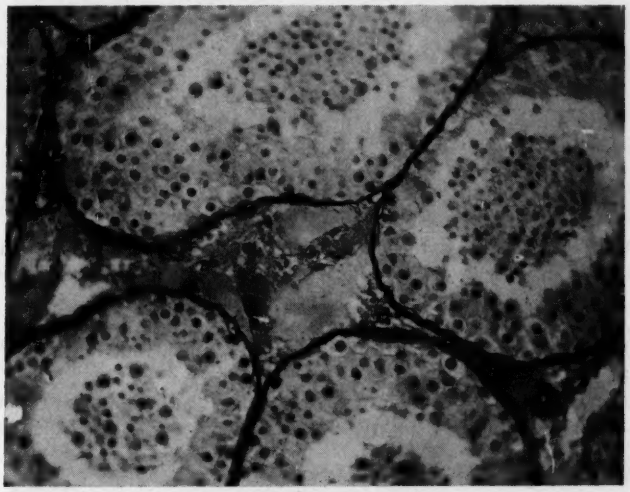
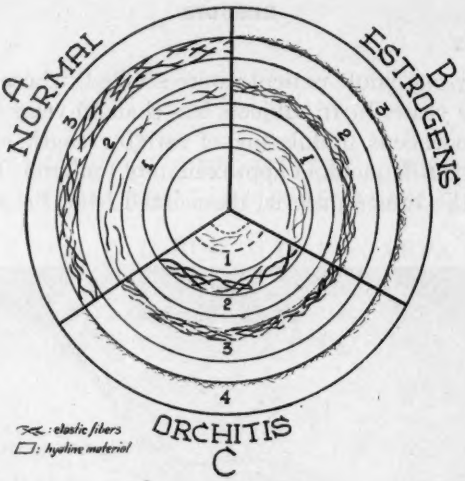


FIG. 1. Section from testis of adult normal male under 50 years of age. (Details described in text.) ×165.

These elastic fibers, although they were always found in close proximity to each other, seemed to be morphologically autonomous and ran in different directions; some encircled the lumen, whereas others took a longitudinal course (Scheme I, Sector A 2).

As with all the other fibrillar components of the tunica propria, elastic fibers varied in number in different subjects, but this did not seem to have any pathologic significance; furthermore, these differences were often observed in one field, where tubules having two or three rows of elastic fibers were adjacent to others with only a few fibers.

In the group of subjects under 50 years of age, the following cases deserve special attention: in 17-year-old subjects the elastic fibers of the tunica propria were fine and sparse in all the tubules (Fig. 2), and in a 48-year-old man they were thicker and more numerous than those observed in the rest of the group.



SCHEME I. Graphic representation of the tunica propria, showing the morphology of the elastic fibers in normal and pathologic conditions. The scheme is divided in three sectors: A, B, and C.

Sector A shows elastic fibers in the testis of normal subjects, classified as follows: 1) prepuberal testes; 2) testes of adult males under the age of 50; and 3) testes of males over 50 years of age.

Sector B shows the changes produced by estrogens in elastic fibers: 1) in tubules with peritubular sclerosis; 2) in tubules with sclero-hyalinosis; and 3) in the totally hyalinized tubule wall.

Sector C shows changes produced in the elastic fibers of the tunica propria of patients suffering from brucellosis orchitis or mumps orchitis: 1) in acute orchitis; 2) in chronic orchitis with peritubular sclerosis; 3) in chronic orchitis with sclero-hyalinized tubule walls; and 4) in chronic orchitis with totally hyalinized tubule walls.

Fifteen normal adults were over 50 years of age. Twelve of them showed an increase in the number, thickness and staining intensity of the elastic fibers (Fig. 3 and Scheme I, Sector A 3).

In 3 subjects over 50 years of age the elastic fibers were similar to those characteristic of the younger age group. When hyalinized tubules were present, the elastic fibers were thick and numerous, and were located just outside the hyaline material.

Newborn. Elastic fibers were not found in the tunica propria of 4 newborn infants (Scheme I. Sector A 1).

Puberty. Eleven subjects were studied, whose ages varied from 10 years to 15 years and 10 months. Testes were classified morphologically as mature, semi-mature, and infantile.

In 10 of these cases the elastic fibers of the tubule wall were sparse, thin,

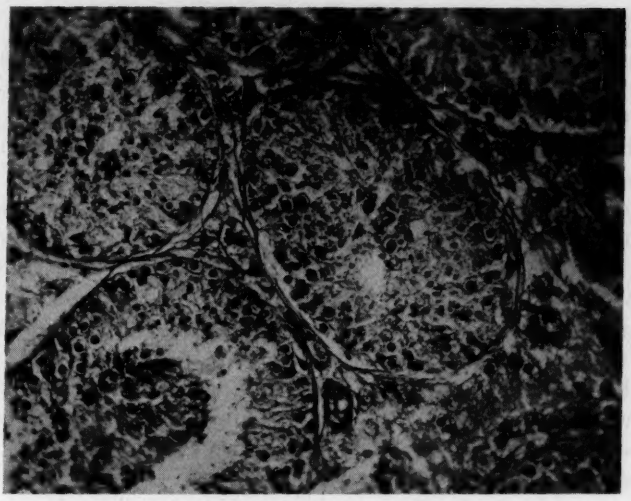


FIG. 2. Section from testis of 17-year-old normal male. Elastic fibers thin and sparse. ×165.

and partially stained; in some tubules they were absent. The more developed elastic fibers were found in the more mature testes. In 1 case in which the testes were of adult maturity morphologically, the elastic fibers had the features described under the heading Normal Adults.

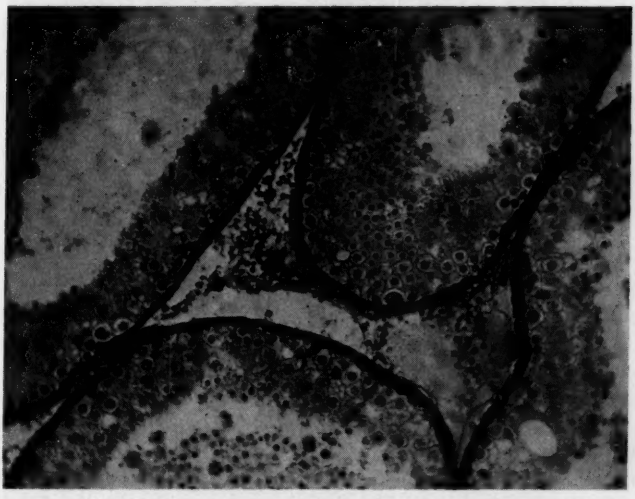


FIG. 3. Section from testis of normal male over 50 years of age. Elastic fibers are increased in number and thickness. ×165.

2. Testes in pathologic conditions

Eunuchoidism with low FSH (5, 6). Eight patients with eunuchoidism and decreased FSH were studied. The ages ranged between 17 and 40 years at the time at which biopsy specimens were obtained. In all these patients, absence of elastic fibers was observed even in the sclero-hyalinized tubules sometimes found in the older age group.

Postpuberal hypogonadism produced by administration of estrogens (7). This study is based on 15 patients 51 to 79 years old with cancer of the prostate gland, who were being treated with estrogen. Twenty-eight testicular biopsies were performed in this group. It was possible to obtain testicular material from 9 patients before beginning estrogen treatment. Each of these 15 subjects underwent one biopsy, and 4 of them, two biopsies. Stilbestrol was administered orally in doses of from 5 to 50 mg. daily. Biopsy was performed at different times during estrogen treatment, the intervals between biopsies being from eight to seventy days.

We have observed that under estrogen therapy involutional changes occur in the tunica propria; these are initiated by reticular sclerosis, which is followed by sclerotic change of the collagen and may reach total hyalinization of the tubule wall.

a) In the tubules with sclerotic changes, the elastic fibers were inside the

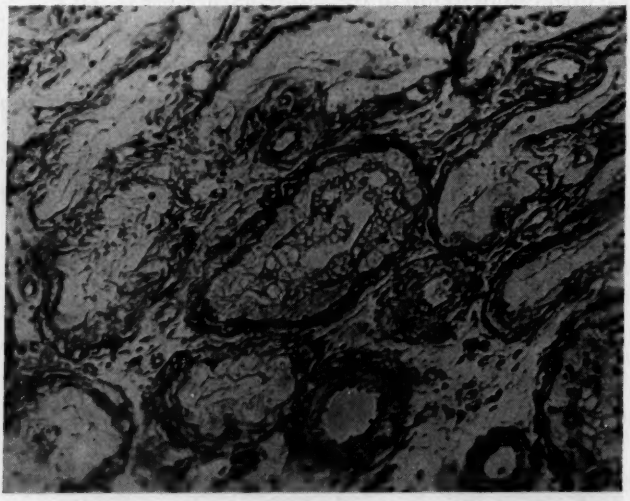


FIG. 4. Section from testis of patient who received 40 mg. of estrogen daily for forty days. Note the sclero-hyalinized tunica propria; elastic fibers are just outside the hyaline material; they are thin but thicker than those observed in the completely hyalinized tubules. In the lower right-hand corner there is a tubule with sclerosis of the wall, showing short, thin elastic fibers. ×165.

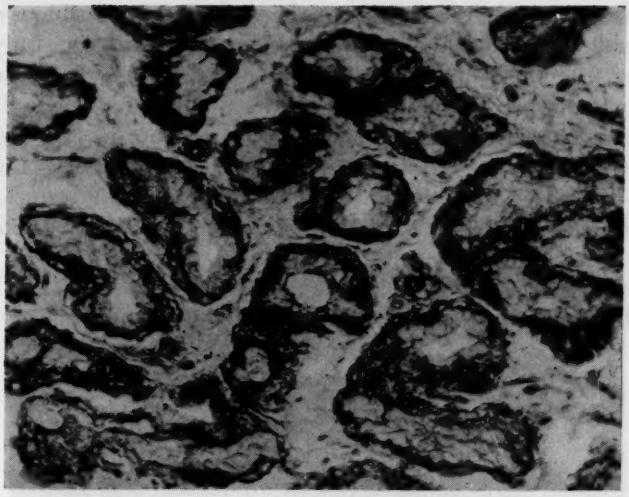


FIG. 5. Section from testis of a patient who received estrogen for two years; most tubules show signs of disappearance but some loose circular nets of elastic fibers remain. ×165.

tunica propria. They were short and thin (Fig. 4 and Scheme I, Sector B 1). This decrease in size of the elastic fibers is more significant if related to the decrease in diameter of the tubules and to the characteristic changes of their age group (*i.e.*, men over 50 years old) which had been apparent in biopsy specimens taken prior to estrogen therapy.

b) In the tubules with sclero-hyalinized tunica propria, the elastic fibers were numerous: they were also located just outside the hyalinized material, forming a net of short, thinned, tapering fibers which were, however, somewhat thicker than those found in (c) (Fig. 4 and Scheme I, Sector B 2).

c) In the tubules with completely hyalinized walls, the elastic fibers were scattered, thin, short and placed just outside the hyalinized material, together with collagen and reticular fibers (Fig. 4 and Scheme I, Sector B 3). In 1 additional case of a man who received estrogen treatment for a period of two years, most tubules showed signs of obliteration, but some loose circular nets of elastic fibers remained (Fig. 5).

In all these estrogen-treated patients, the morphologic changes of the elastic fibers did not appear early in treatment (*i.e.*, biopsy specimens taken after eight, eleven and thirteen days). In biopsies performed after twenty-three days of estrogen therapy, the first alterations observed were shortening and thinning of fibers persisting inside the tunica propria.

Eunuchoidism with high FSH (5) or functional prepuberal castration (8).

Two cases were studied whose ages at the time of testicular biopsy were 21 and 26 years. The material obtained by biopsy for histologic study was, in both cases, one third of the whole organ; nevertheless, no tubules were found—only fibrous material.

Klinefelter's syndrome (9). There were 13 patients in this group, and their ages ranged from 15 to 40 years. Absence of elastic fibers was apparent in all the hyalinized tubules. In 6 patients some tubules were seen having thin, sparse elastic fibers and these tubules also contained trophic Sertoli cells and spermatocytes.

Arrest of spermatogenesis. Nine such patients were studied (10). The ages of the subjects ranged from 26 to 38 years. They were primarily sterility

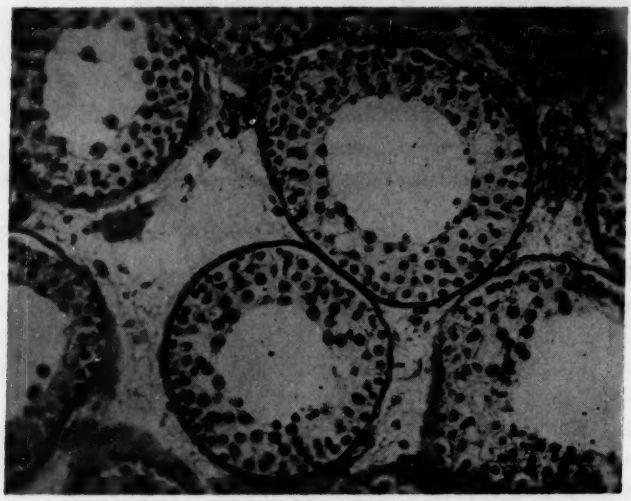


FIG. 6. Section of testis showing spermatogenesis. Elastic fibers are similar to those of a normal adult male under 50 years of age. ×165.

problems and in all of them spermograms were abnormal. The physical examination gave negative results, and urinary 17-ketosteroids and FSH were normal. Testicular biopsies (Fig. 6) showed a normal histologic picture, except for arrest of maturation of spermatogenesis. The elastic fibers in the tunica propria were normal.

Syndrome of absence of germ cells. Six subjects were studied; their ages ranged from 21 to 51 years. In the tubules containing only Sertoli cells (Fig. 7), elastic fibers were found in several rows of thick, long fibers with strong staining reaction. As the tubules had become narrower, it remains to be shown whether the increase in the elastic fibers was absolute or rela-

tive. When sclero-hyalinized tubules occurred, the somewhat thin elastic fibers were located just outside the hyaline material.

In tubules in which the germinal epithelium still persisted, elastic fibers resembled those found in normal adults over 50 years of age.

True precocious puberty. Two patients were studied. In one of them, puberty had begun at the age of 5 years. Biopsy was performed at 6 years of age. The tunica propria of all the tubules contained thin, long, undulating elastic fibers. In the other patient, puberty began at 8 years of age and

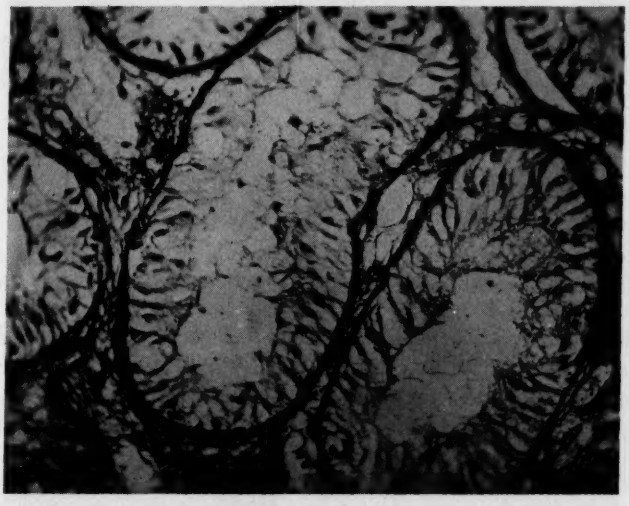


FIG. 7. Section from testis of a patient who had no germ cells. In the tunica propria are several rows of thick, long elastic fibers with strong staining reaction. ×165.

biopsy was performed when the patient was 11 years old; elastic fibers were found and they were similar to those of the normal adult under 50 years of age.

Brucellosis with epididymitis and orchiepididymitis (11). Thirteen patients suffering from brucellosis were studied, who presented symptoms of unilateral or bilateral epididymitis and orchiepididymitis in acute, subacute and chronic forms.

Sixteen testicular biopsies were performed, of which 11 were bilateral; thus the number of testes examined was 27. Biopsies were performed at periods ranging from eleven days to seven years after the genital localization of the disease.

In acute orchitis, elastic fibers were dissociated, with a tendency to be greatly reduced in number or absent (Fig. 8 and Scheme I, Sector C 1).

In subacute orchitis, the elastic fibers were thin and short. In the cases

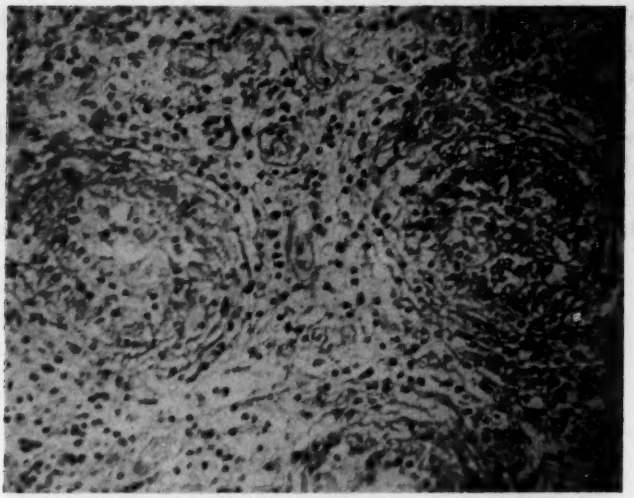


FIG. 8. Section from testis in a case of acute brucellosis orchitis. Elastic fibers are dissociated and show tendency to disappear. Poor staining reaction. ×165.

of chronic orchitis, with peritubular sclerosis or sclero-hyalinosis, it was found that: a) in peritubular sclerosis, the elastic fibers were increased in number and thickness; they were long and located inside the tunica propria (Fig. 9 and Scheme I, Sector C 2), b) in sclero-hyalinized tubules, elas-

FIG. 9. Section from testis in a case of chronic brucellosis orchitis. In a group of tubules with sclerotic walls, elastic fibers are long and have increased in number and thickness. ×165.

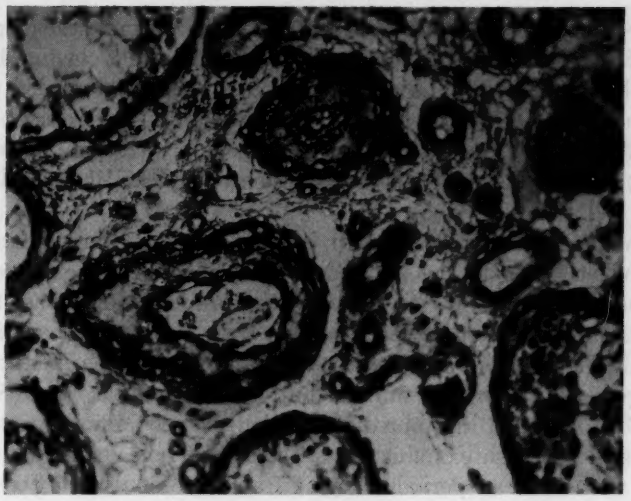


FIG. 10. Section from testis in a case of chronic brucellosis orchitis. In the center may be seen two completely hyalinized tubules; the elastic fibers are thin, interwoven in the form of a net, and are placed just outside the hyaline material. In the lower right-hand corner a sclero-hyalinized tubule may be seen; the hyalinization is just beginning; the elastic fibers are thick and just outside the hyaline material. ×165.

FIG. 11. Section from testis in a case of chronic mumps orchitis. In one tubule showing arrest of spermatogenesis (lower right-hand corner), it is possible to observe an increase in the number of elastic fibers inside the tunica propria. In the upper part, four completely hyalinized tubules may be seen. Elastic fibers are just outside the hyaline material, forming a net of fibers which may be thick or thin. ×165.

tic fibers were thick and just outside the hyalinized material (Fig. 10 and Scheme I, Sector C 3), c) in tubules entirely hyalinized, elastic fibers were outside the hyalinized material, but they were thin and tended to disappear (Scheme I, Sector C 4), and d) in tubules without sclerosis of the walls but with arrest of maturation of spermatogenesis, elastic fibers were normal; in a few tubules, however, they appeared to have increased in number and thickness.

The foregoing findings can be applied to all cases of chronic brucellosis-orchitis, whether the lesions be mild or intense, localized or diffuse.

Mumps orchitis. The elastic tissue of the tunica propria was studied in 7 patients suffering from mumps orchitis. In 1, biopsy was made four days after genital localization during acute orchitis; the elastic fibers of the tunica propria were normal in number but they were dissociated (Scheme I, Sector C 1) and had an anomalous bluish-green staining reaction. The physical and histologic examination of the other 6 revealed chronic orchitis. *Biopsies* were made at periods varying from two months to twenty years after genital localization. In these cases of chronic mumps orchitis, changes very similar to those seen in chronic brucellosis orchitis were seen (Fig. 11).

a) In tubules with peritubular sclerosis, there was an increase in the number of thicker elastic fibers located inside the tunica propria (Scheme I, Sector C 2).

b) In tubules which were entirely hyalinized, elastic fibers were just outside the hyalinized material, and were in the form of a net. In some tubules elastic fibers were thick, numerous and intensely stained. In the walls of other tubules, elastic fibers were short and thin (Scheme I, Sector C 4).

c) When hyalinized tubules were in an advanced state of dissolution, elastic fibers tended to disappear, or showed up only in clusters.

d) In the tunica propria of tubules showing arrested spermatogenesis, thickened elastic fibers were frequently found.

DISCUSSION

The elastic tissue is one of the fibrillar constituents of the interstitial connective tissue; it possesses morphologic and staining individuality, a special chemical composition (Lansing (12)), and an ultra-fine morphology, very different from those of collagenous and reticular fibers. To this must be added the difference revealed under the action of collagenolytic enzymes, for elastic fibers are dissolved when treated with elastinase (Balo and Banga (13)).

During this study, we were impressed by the fact that elastic fibers were absent from the tunica propria of the prepuberal testes; the fibers appeared at puberty and attained adult features just after puberty.

This development occurred simultaneously with the maturation of the

tubular content (germinal epithelium and Sertoli cells) and with the appearance of abundant trophic Leydig cells in the intertubular connective tissue.

However, testicular material which we obtained from patients during puberty demonstrated that groups of trophic Leydig cells may exist even though the elastic fibers of the tunica propria have not yet completely developed.

We confirmed Sniffen's observation (1), that in a high percentage of adult normal males over 50 years of age there is a marked increase in the number and thickness of the elastic fibers of the tunica propria.

It is certain that some factor or factors which determine the appearance of elastic fibers begin their activity at normal puberty, at a time in the development of the individual in which numerous influences are set in motion, *e.g.*, those of pituitary gonadotropins, testicular androgens, or adrenocortical hormones. These are intimately connected with the development and morphology of the connective tissue.

Therefore, it can be assumed that all these factors might influence the equilibrium between the cells and the amorphous substance of the tunica propria— an equilibrium which might determine the appearance of elastic fibers. However, the assumed intervention of fibroblasts in the production of elastic fibers should be kept in mind. Observation of the morphologic and histochemical changes produced in fibroblasts of the tunica propria by various stimulants (14, 15) leads one to believe that they are markedly different from the other testicular and body fibroblasts. They may have an important function to perform, namely, to maintain an adequate balance between the fibrillar and amorphous components of the wall. Any abnormal stimulant, either hormonal, infectious or of some other nature, affecting the fibroblasts would also influence maintenance and structure of the tubular wall, thus altering the elastic fibers.

It has not been possible for us to ascertain which of the following factors is the more important for the development and preservation of the elastic fibers of the tubular wall: 1) adequate function of the pituitary-gonadotropins, or 2) the presence of trophic and secretory Leydig cells. It should be pointed out that elastic fibers were not present in the testes of patients with hypogonadotropic hypogonadism, and that in the testes of patients with Klinefelter's syndrome (increased pituitary gonadotropins) elastic fibers were found only in the tunica propria of the tubules which had reached a certain degree of maturation. In a previous study on this syndrome, we pointed out (15) that the better preserved tubules were usually the ones near the few groups of trophic and secretory Leydig cells.

The alterations caused in the elastic fibers by chronic brucellosis orchitis are very similar to those caused by mumps orchitis.

The injury caused by the administration of estrogens is different from that caused by orchitis in that, during the first stage of fibrillar thickening in the tunica propria, the elastic fibers become thinner instead of thicker.

Although we have not been able to ascertain the functional or genetic significance of the elastic fibers of the tunica propria, the study of elastic fibers under the various described conditions has enabled us to establish in part, whether or not alterations suffered by the testes have occurred before or after puberty.

SUMMARY

The morphology of the elastic fibers of the tunica propria in normal testes and in pathologic conditions has been studied.

Elastic fibers were absent from the tunica propria of prepuberal testes; they appeared at the time of puberty and attained adult features just after puberty.

In cases of eunuchoidism with low FSH excretion, the elastic fibers were absent; in postpuberal hypogonadism produced by administration of estrogen, they were short and thin; they were found in 6 of 13 cases of Klinefelter's syndrome; in true precocious puberty they resembled the fibers seen in the postpuberal state; they were normal in cases of arrested spermatogenesis; and they were found in several rows of thick, long fibers with strong staining reaction in the syndrome associated with absence of germ cells.

The possible factors determining the appearance and maintenance of elastic fibers are discussed.

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A CORTISONE-RESISTANT ABNORMALITY IN THE DIURETIC RESPONSE TO INGESTED WATER IN PRIMARY MYXEDEMA

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IN NORMAL humans the ingestion of 20 milliliters of water per kilogram of body weight evokes a characteristic water diuresis. After a slight delay, urine flow increases and peak flows are achieved within one hour, at which time a copious dilute urine is excreted. In primary hypoadrenalism the excretion of water after ingesting such a water load is delayed (1, 2). The following report indicates that a similar abnormality in the diuretic response to ingested water is associated with untreated primary idiopathic myxedema.

In hypoadrenalism treatment with cortisone reinstates a normal diuretic response to a water load (1, 3). Studies are presented to show that cortisone therapy does not establish a normal diuretic response in untreated primary myxedema, whereas thyroid therapy does in association with the change to the euthyroid state. Studies in postoperative myxedema and in postirradiation myxedema are also reported.

MATERIALS AND METHODS

Eleven patients (9 female, 2 male) with primary myxedema (8 idiopathic, 2 postoperative, 1 postirradiation) were given the standard water test as described by Robinson, Power and Kepler (2). The results were evaluated in the usual manner, and in addition the percentage of the water load excreted in the four-hour period was calculated. To obviate the factor of intestinal absorption, on three occasions the water load was given intravenously as 5 per cent glucose in distilled water.

The foregoing procedures were carried out prior to therapy and after administering 1) cortisone, 2) thyroid, and 3) thyroid and cortisone, in the dosages indicated in Table 1.

RESULTS

Primary idiopathic myxedema

As shown in Table 1, all of the 8 patients with primary idiopathic myxedema showed a delay in water diuresis compared to the normal controls. Myxedematous patients excreted an average of about 42 per cent of the

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TABLE 1

	URINARY VOLUMES - ml					SPECIFIC GRAVITY OF URINE				% EXCRETION OF WATER	"A" VALUE	ROUTE OF ADMINISTRATION	MEDICATION
	Night Urine	1	2	3	4	1	2	3	4				
		(Hrs. After Water)				(Hrs. After Water)							
PRIMARY MYXEDEMA - IDIOPATHIC													
W.V. - 1	603	43	150	155	175	1.009	1.009	1.009	1.010	43	6	Oral	None
- 3	1560	0	197	226	127	-	1.005	1.005	1.004	43	4	Oral	None
- 3	767	60	176	197	215	1.013	1.006	1.006	1.005	49	9	Oral	Cortisone - 100mg. daily - 10 days
- 4	385	48	138	160	152	1.015	1.005	-	1 005	38	8	Oral	Thyroid - 60mg. daily - 6 wks.
- 5	475	392	483	122	30	1.015	1.010	1.012	-	82	67	Oral	Thyroid - 60mg. daily - 6 wks. plus Cortisone - 100mg. daily - 10 days
P.T. - 1	-	80	221	177	195	1.009	1.004	1.003	1.003	44	-	Oral	None
- 2	595	135	375	210	138	1.008	1.004	1.003	1.004	45	23	Oral	None
- 3	600	115	332	189	115	1.007	1.003	1.003	1.004	49	11	Oral	Cortisone - 300mg. 2 hrs. previous to test
L.T. - 1	350	40	51	67	59	1.014	1.014	1.013	1.013	23	-	Oral	None
- 2	750	195	271	245	56	1.015	1.008	1.007	1.007	67	-	I.V.	None
- 3	680	110	290	282	165	1.006	1.006	1 005	1.007	74	-	I.V.	Cortisone - 200mg. 2 hrs. previous to test
E.R. - 1	750	50	85	305	326	1.019	1.015	1.007	1.006	49	4	Oral	None
- 2	305	135	415	425	303	1.016	1.003	1.001	1.003	81	101	Oral	Thyroid - 15mg. daily - 10 days Cortisone - 100mg. daily - last 5 days
- 3	-	195	462	486	390	1.008	1.003	1.003	1.004	105	-	Oral	Thyroid - 65mg. daily - 3 months
M.B - 1	243	105	253	216	95	1.014	1.007	1.005	1.005	40	-	Oral	None
- 2	345	62	258	345	304	1.013	1.007	1.006	1.007	68	-	Oral	Thyroid - 65mg. daily - 3 months
O.W. - 1	185	213	224	75	93	1.012	1.007	1.006	1.007	43	-	I.V.	None
- 2	150	31	115	340	151	-	1.005	1.004	1 006	42	-	Oral	None
- 3	-	351	554	345	198	1.005	1.004	1.004	1.001	96	-	I.V.	Thyroid - 48mg. daily - 3 months
E.W. - 1	275	85	130	165	188	1.010	1.003	1.002	1.003	42	11	Oral	None
- 2	360	365	700	485	38	1.004	1.004	1.004	1.020	118	-	Oral	Cortisone - 100mg. - 12 hrs. and 2 hrs. before
B.P. - 1	475	0	245	170	290	-	1.007	1.004	1.007	47	-	Oral	None
- 2	460	145	230	310	220	1.009	1.002	1.002	1.003	59	-	Oral	Cortisone - 100mg. - 12 hrs. and 2 hrs. before
PRIMARY MYXEDEMA - POST OPERATIVE													
B.P. - 1	450	52	119	115	93	1.014	1.019	1.020	1.019	25	-	I.V.	None
- 2	350	52	193	195	192	1.016	1.006	1.006	1.005	40	-	I.V.	Cortisone - 100mg. daily - 10 days
- 3	165	27	335	305	250	1.020	1.006	1.005	1.007	68	-	Oral	Thyroid - 65mg. - 6 months 130mg. - 3 months
L.S. - 1	380	40	180	55	26	1.005	1.005	1.007	1.010	33	16	Oral	None
- 2	388	40	141	58	30		1.005	1.010	1 012	30	11	Oral	Cortisone - 100mg. - 8 hrs. and 2 hrs. previous to test
PRIMARY MYXEDEMA - POST RADIATION													
E.G. - 1	190	130	360	305	220	1.010	1.005	1.004	1.006	80	-	Oral	None
- 2	362	292	332	384	195	1.004	1.001	1.001	1.001	93	-	Oral	None
NORMALS													
R.W.	-	360	385	375	210	1.002	1.001	1.002	1.005	104	-	Oral	
D.R.	-	60	635	431	22	1.010	1.002	1.001	1.024	77	-	Oral	
W.O.	-	493	497	113	50	1.009	1.002	1.005	1.014	82	-	Oral	
R.J.	-	432	498	297	53	1.010	1.004	1.005	1.020	85	-	Oral	
R.G	-	447	727	115	50	1.008	1.004	1.012	1.021	87	-	Oral	
R.T.	-	218	563	362	22	1.012	1.005	1.006	1.020	103	-	Oral	

ingested water over a four-hour period as compared to an average excretion of about 90 per cent in the normal controls.

Six of the 8 patients had an abnormal response to Part I of the Robinson-Power-Kepler water test. Part II of this test was carried out in 4 of the 6 patients and the results were positive in all 4, *i.e.*, the value of "A" was less than 25.

Four out of 5 patients given two doses of oral cortisone, in a dose which corrects the delayed water excretion in hypoadrenalism (4), continued to exhibit an abnormal delay in water diuresis. Cortisone likewise failed to correct the abnormal Robinson-Power-Kepler test findings. One patient

(W. V.), given 100 mg. of cortisone daily for ten days, also failed to show a normal diuretic response or a normal result with the Robinson-Power-Kepler test. In 1 patient (E. W.) the administration of cortisone corrected both the abnormalities.

Two patients (E.R. and O.W.), having attained the euthyroid state with thyroid therapy, responded normally to ingested water.

Two patients (E.R. and W.V.), receiving thyroid but still hypothyroid, showed a normal diuretic response when given oral cortisone in addition to the thyroid.

Two untreated patients, given the test load intravenously as 5 per cent glucose in distilled water, showed the same abnormal delay in water excretion.

Postoperative and radiation myxedema

The 2 patients with postoperative myxedema of at least two years' duration showed the same abnormal delay in water excretion and abnormal results with the Robinson-Power-Kepler test as the patients with primary myxedema.

The 1 patient with myxedema of only three months' duration, secondary to radioactive iodine therapy, showed no abnormality in water excretion and the Robinson-Power-Kepler test results were normal.

DISCUSSION

These data show that excretion of a water load is impaired in long-standing, untreated, primary myxedema. Following oral administration of water, an unusually low fraction of the water load is excreted over a four-hour period, and the Robinson-Power-Kepler test findings are abnormal. Depressed intestinal absorption of water, a potential factor in the foregoing results, does not seem to have significance in the delayed diuretic response, since the same volume of water administered intravenously as a 5 per cent glucose solution does not materially improve the diuretic response.

It has been demonstrated that primary myxedema may be associated with hypoadrenalism (5). The abnormal water excretion was not corrected in 9 of 10 myxedematous patients by the administration of cortisone in doses adequate to correct the delayed diuretic response in hypoadrenalism. The single patient in whom cortisone was efficacious had not received thyroid during the year prior to the present study, although she had received thyroid during the four previous years. All other patients had received no thyroid therapy prior to the present studies.

The results in the 1 patient with postirradiation myxedema of only three months' duration suggest that the duration of the myxedematous state

may be a factor in this abnormality. All of the other patients had myxedema of at least two years' duration.

Water excretion in the normal human is influenced by the rate of glomerular filtration and the rate of reabsorption in the tubule. The latter factor, in turn, is regulated by solute reabsorption and the activity of the neurohypophysis (6).

It has been reported in both myxedema (7, 8) and hypoadrenalism (4, 9) that the glomerular filtration is depressed. Cortisone administered to patients with hypoadrenalism causes an increase in glomerular filtration rate, and Burston and Garrod (4) suggest that a reduction in this function is one factor in the depressed water excretion in hypoadrenalism. In myxedematous patients, the glomerular filtration rate is also decreased below the normal range, and treatment with desiccated thyroid restores the rate to more normal values (7). Several studies suggest a relationship between the thyroid and the neurohypophysis in water metabolism (10, 11).

These data do not allow an interpretation of the disturbed mechanisms for the control of water excretion in primary myxedema. All that can be said is that the administration of thyroid with attainment of the euthyroid state establishes a normal diuretic response to a water load in patients with primary myxedema.

CONCLUSIONS

1. Patients with untreated primary myxedema of long duration have an abnormal diuretic response to the ingestion of a water load.

2. This abnormality is not corrected by cortisone but is corrected when the euthyroid state is attained following the administration of thyroid.

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THE RATE OF DISAPPEARANCE OF EXOGENOUS PROGESTERONE FROM THE BLOOD*†

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STUDIES on the metabolism and utilization of progesterone have depended primarily on the recovery of pregnanediol from the urine. Since urinary pregnanediol accounts for only about 20 per cent of the progesterone it is obvious that such studies can only approximate the changes in the hormonal level of the blood. It has even been suggested that there is no quantitative relationship between the circulating progesterone and the pregnanediol excreted (1, 2, 3). In addition, it has been demonstrated that pregnanediol may arise from desoxycorticosterone (4, 5).

With the development of a highly sensitive and specific assay for progestational activity (6, 7, 8) it became possible to examine the levels of progesterone[1] in the blood. The present study is concerned with the rate of disappearance of progesterone from the blood following intramuscular and intravenous injections of physiologic amounts of the hormone. In addition, the roles of the liver and kidney were evaluated in the initial loss of progesterone from the blood.

MATERIAL AND METHODS

Mature male or female rabbits of the grey Chinchilla strain were used in this study. All the rabbits were gonadectomized at least one week prior to treatment and kept at 72° F. with food and water supplied *ad libitum*. Nephrectomies were performed under Nembutal anesthesia supplemented by ether, and the dorso-lateral approach was used. In all instances both kidneys were removed simultaneously and the animals were permitted to recover from the anesthesia before treatment. In the experiments involving hepatectomy, the liver was excluded by ligation of its total blood supply. Traumatic shock was induced by direct manipulation of the intestine of the animal for five minutes.

The progesterone was dissolved in sesame oil for the intramuscular injections and in

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[1] Although the Hooker-Forbes method measures total progestational activity (progestin), rather than progesterone specifically, we assume the activity is that of progesterone, at least in the instances in which progesterone proper was injected into the animals.

a propylene glycol-albumin solution (9) for the intravenous injections. In one instance, a microcrystalline suspension in 10 per cent alcohol was used for intravenous injection. Plasma volumes were determined by the dye dilution method (10), using Evans Blue, T-1824. In all experiments, with the exception of intramuscular injections, the rabbits received an amount of progesterone calculated to give a concentration of 10 micrograms of hormone per milliliter of plasma. The rabbits were bled from the marginal vein of one ear and the injections were made into the marginal vein of the contralateral ear.

Collected sera were promptly frozen and kept at −20° C. until assayed. All assays for free progesterone[1] were carried out on the untreated serum, according to the technique of Hooker and Forbes (1947), using Rockland-Swiss mice. The concentration of protein-bound progesterone, was determined after first hydrolyzing the serum at pH 1 for six hours and then extracting the hydrolysate with an ethanol-ether mixture (11, 12). The extract was then evaporated to dryness and dissolved in sesame oil for assay. Both the muscle and fat were homogenized in a mechanical grinder and the homogenates extracted with ether. The ether was then removed *in vacuo* and the extract dissolved in sesame oil.

EXPERIMENTAL

A. *Disappearance of progesterone following intramuscular injection*

Three castrated rabbits were injected intramuscularly with progesterone and the blood level of the hormone was determined over a twelve-hour period (Fig. 1). It may be seen that treatment with 10 mg. of progesterone

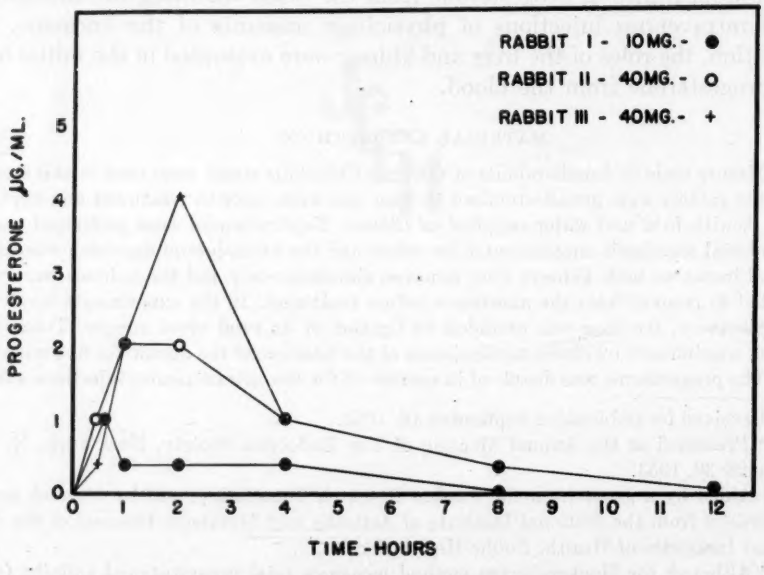

FIG. 1. The rate of appearance and disappearance of progesterone from the blood serum following an intramuscular injection of the hormone. A dose of 40 mg. was required to produce an appreciable concentration of the hormone in the blood.

produced a maximum concentration of 1 μg. per ml. of serum in thirty minutes, which dropped to 0.3 μg. per ml. in one hour. The hormone was maintained for several hours at this level and then disappeared completely by the eighth hour.

Treatment with 40 mg. of progesterone caused a peak of 2 μg. per ml. of serum at one hour in one rabbit and a peak of 4 μg. per ml. in two hours in the second animal. In both instances the concentration fell to 1 μg. per ml. by the fourth hour and was no longer detectable in the body at twelve hours after injection.

B. *Disappearance of progesterone following intravenous injection*

In these experiments and all subsequent ones, plasma volumes were determined initially and the animals were injected with a quantity of progesterone calculated to produce a concentration of 10 μg. per ml. of plasma. This theoretical value was taken as the initial concentration and the rate of disappearance was based on this value.

The data in Figure 2 represent the rate of disappearance of progesterone

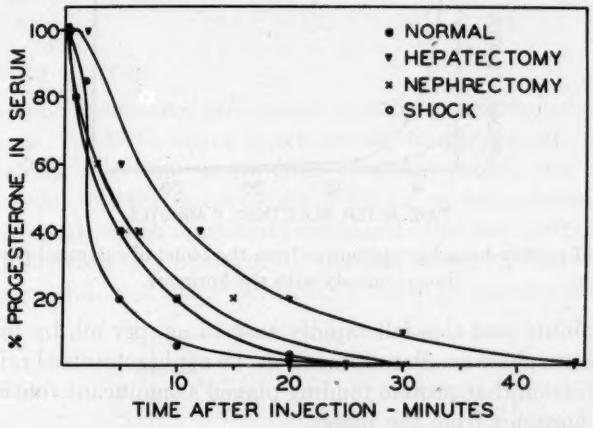

FIG. 2. Rate of disappearance of progesterone from the blood of rabbits following intravenous injections of the hormone. The control castrated rabbits showed the most rapid loss, whereas the nephrectomized animals and the animal exposed to traumatic shock showed nearly identical rates, both of which were slower than that seen in the controls. The hepatectomized rabbits showed the greatest delay in rate of loss of the hormone.

from the blood of a) castrated control rabbits, b) rabbits subjected to traumatic shock, c) nephrectomized rabbits and d) hepatectomized rabbits. It is apparent from these data that removal of the liver or kidneys delayed the rate of disappearance of the hormone. Whereas 60 per cent of the hor-

mone was lost from the blood of castrated control animals within three minutes, an equal loss required five and a half minutes in the case of the nephrectomized animals and nine minutes in the case of the hepatectomized rabbits. It is of interest to note that the shock-control rabbit showed a disappearance rate almost identical with that observed in the nephrectomized animals.

C. *Protein-bound progesterone*

Analyses of the blood serum for concentration of protein-bound progesterone failed to reveal any significant rise. The data in Figure 3 indicate a rapid loss of total progesterone following intravenous injection. The protein-bound progesterone constituted only a small part of the total circulating hormone. An initial peak of 1.5 μg. per ml. was noted in approxi-

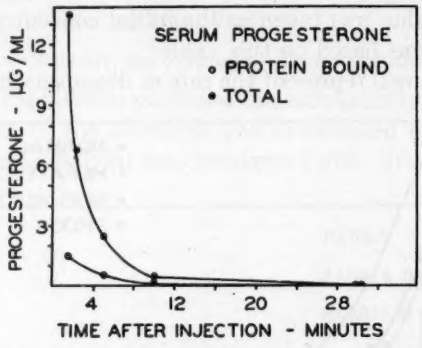

FIG. 3. Loss of protein-bound progesterone from the blood of castrated rabbits injected intravenously with the hormone.

mately a minute and this fell rapidly to 0.15 μg. per ml. by ten minutes. Comparable results were also obtained in the nephrectomized rabbit. There was no indication that protein binding played a significant role in the rapid loss of the hormone from the blood.

D. *Progesterone in fat and muscle*

Analyses of fat and muscle revealed the presence of progesterone following injection of the hormone. A concentration of 100 μg. per 100 grams of fat was noted in fifteen minutes. This level increased to 230 μg. by thirty minutes and then fell to the zero level within one hour (Table 1). A similar curve was noted for muscle, except that this tissue contained only one tenth the concentration seen in the fat. No progesterone was found in the fat or muscle of the untreated, castrated control rabbits (Table 1).

In the course of these experiments it was possible for us to assay the

TABLE 1. PROGESTERONE[1] IN THE TISSUES OF THE CASTRATED RABBIT FOLLOWING INTRAVENOUS INJECTION OF THE HORMONE

Rabbit No.	Body wt. (kg.)	Plasma volume (ml.)	Treatment	Time after injection (mins.)	Progesterone[1] in fat (μg./100 Gm.)	Progesterone[1] in muscle (μg./100 Gm.)
V	2.2	—	None	—	0	0
VI	4.15	250	2.5 mg. progesterone I.V.	15	100	10
VII	3.3	230	2.3 mg. progesterone I.V.	30	230	3
VIII	4.3	270	2.7 mg. progesterone I.V.	60	0	0

level of progesterone in the adipose tissue of a castrated rabbit that had been injected intramuscularly with 1 mg. of progesterone daily for four hundred days. This animals showed a concentration of 320 μg. per 100 grams of fat.

E. *Incubation of serum*

Progesterone levels were determined at definite intervals during incubation of serum at 37°C. Since progesterone disappears rapidly from the blood following injection, serum from pregnant rabbits was used in this experiment. An initial concentration of 6.0 μg. of progesterone per ml. of serum was found, which remained unchanged after one hour of incubation (Fig. 4). Following two hours of incubation, however, the concentration fell to 5 μg. per ml., and after four hours a loss of 95 per cent of the activity

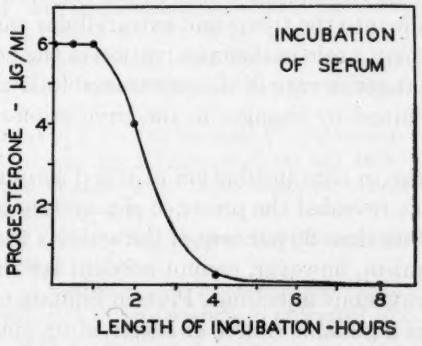

FIG. 4. Loss of progesterone following *in vitro* incubation of the serum at 37° C.

was noted. No progestational activity was detectable in the serum incubated for eight hours.

DISCUSSION

In general these results agree with the report of Haskins (13), who showed by spectrophotometric analysis a 60 per cent loss of progesterone from the blood within a few minutes. Such a rapid loss of hormone from the circulating blood appears to hold for all the steroids that have been examined thus far. Both Haskins (13) and West (14) have shown this to be also true for testosterone. In the present study, 92 per cent of the progesterone was lost from the blood within ten minutes following intravenous injection of the hormone. Following nephrectomy, a reduced rate of loss was observed, so that only 70 per cent was lost within ten minutes. In 1950, Forbes, Hooker and Pfeiffer indicated that passage of blood through the kidney caused a loss of 0.6 mg. of progesterone from the plasma per minute. In these experiments removal of the kidneys caused some delay in the rate of disappearance of the hormone but the change in rate was not very great.

Similarly, the liver has been suggested as a site of conversion or inactivation. Intrasplenic or mesenteric implantation of ovaries or of progesterone results in an inactivation of the hormone (16–19). Ligation of the blood supply to the liver has been shown to cause a decrease of only 0.8 per cent in the rate of disappearance of progesterone (20), whereas Haskins (13) has reported a highly significant decrease in the loss of progesterone from the blood of a hepatectomized rabbit. As in the case of our nephrectomized rabbits, hepatectomized animals showed a decreased rate of loss, so that only 60 per cent was lost in ten minutes. However, control experiments with traumatized rabbits also showed a delay in rate of loss of the hormone from the blood. One can only conclude at this time, that the rapid loss of progesterone from the circulation is due to the rapid diffusion of the relatively small molecule into the tissue and extracellular spaces. That the kidney and liver may play a role in the inactivation of the hormone is not to be denied, but the changes in rate of disappearance following removal of the organs can be explained by changes in the circulation due to the surgical trauma.

In our studies, the *in vitro* incubation of blood serum containing a high titer of progesterone revealed the presence of a system for the inactivation of the hormone. More than 90 per cent of the activity was destroyed in four hours. This mechanism, however, cannot account for the rapid disappearance following intravenous injections. Protein binding of the hormone was also investigated as a possible source of inactivation and again the present data do not support the concept that protein binding is involved in the initial loss of the hormone. Analyses failed to reveal any significant increase

in the protein bound progesterone of the blood.

Further evidence for the concept that the hormone diffuses rapidly out of the circulation may be seen in the data on progesterone levels in fat. The hormone can be easily detected within fifteen minutes after administration and reaches a peak by thirty minutes. However, within sixty minutes the hormone is no longer present as such in the fat.

The present data concur with the results of West (1951) on testosterone and Haskins (1950) on progesterone, and indicate that progesterone rapidly diffuses from the circulation into the tissues, particularly the fat.

SUMMARY AND CONCLUSIONS

Intramuscular injection of progesterone produces a maximum concentration in the blood within thirty minutes to two hours, depending upon the dosage. Ten minutes after an intravenous injection of the hormone, 90 per cent of the activity was lost in the normal rabbit, 70 per cent in the nephrectomized, 70 per cent in the traumatized and 60 per cent in the hepatectomized animal. It was pointed out that plasma protein binding and inactivation in the blood stream were not factors in the rapid loss of the hormone. It was concluded from determinations of the progesterone content of tissues, especially fat, that the initial and rapid loss of the hormone is due to a diffusion from the blood into the tissues.

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ADDISON'S DISEASE IN A BOY WITH HYPOPARATHYROIDISM*

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INTRODUCTION

TWO and possibly 3 cases of hypoparathyroidism with Addison's disease have been presented in the literature. Leonard (1) described a patient who had hypoparathyroidism at the age of 3 years. Superimposed adrenal insufficiency occurred at the age of 10½ years. Postmortem examination verified the clinical impression of hypoparathyroidism and Addison's disease. The second patient, an 8-year-old girl with moniliasis, adrenal insufficiency and superimposed hypoparathyroidism, was mentioned briefly in a preliminary report by Talbot *et al.* (2). In addition, Sutphin, Albright and McCune (3) presented what seems to be a third patient with these two diseases. Their patient had Addison's disease and tetany with low serum concentrations of calcium and phosphorus. Because of a history of pleural infusion and a positive tuberculin reaction, they suggested that this was probably the result of tuberculous infection. The patient reported here was a 10-year-old boy in whom Addison's disease developed after he had had signs of hypoparathyroidism for five years.

It is hoped that this report will shed some light on the etiology of this syndrome and provide information concerning the effect of adrenal insufficiency upon the altered metabolism of hypoparathyroidism treated with vitamin D.

CASE HISTORY

The patient (**Y. S.** #Q 8088) was admitted to the hospital with the chief complaint of alopecia, which was first noted when he was 5½ years old, one month after a tonsillectomy. The past history was essentially noncontributory. The patient, the only child in the family, had had a skull fracture at the age of 3½ years, without known sequelae. For four and a half years, he had had many exacerbations and remissions of his alopecia. His disease was diagnosed as alopecia areata by the physicians who saw him during this

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time. The disease involved his eyebrows and eyelashes as well as the hair on his scalp. He had been treated with many vitamin preparations, shampoos and tonics, and at one period received iodine by mouth. For at least one year before entry, the patient had frequent inflammation of the eyes and questionable photophobia. At no time had he any overt fits or loss of consciousness.

Physical examination on admission revealed a well developed and well nourished 10-year-old white boy in no acute distress. Temperature, pulse and respirations were normal. He was 138 cm. tall and weighed 33 Kg. His skin was warm and dry and had a rather sallow appearance. There were several large confluent areas of baldness. A few hairs comprised the eyebrows and eyelashes.

Several molars showed extensive caries. There was an apparent enamel defect of his incisors. His toenails were short, thick and brittle; they were opaque and ridged. The fingernails did not seem to be as markedly involved. There was slight photophobia and moderate injection of his conjunctivae and sclerae. Slit-lamp examination of the eyes was not carried out. The rest of the physical examination was noncontributory.

Results of routine urinalysis and blood counts were all within normal limits. The Kahn reaction was negative. Tuberculin 1:1000 and 1:100, and histoplasmin skin tests gave negative reactions. Monilia could not be cultured from the nails or mucous membranes in several attempts. X-ray examination revealed no bony abnormalities. The serum calcium level on admission was 8.9 mg., serum phosphorus 10 mg., and nonprotein nitrogen 30 mg. per 100 cc.; the levels of serum sodium, chloride and potassium were 148, 96 and 4.6 mEq./L., respectively.

Phosphate clearance studies revealed very low excretion and high tubular reabsorption of phosphate, even after an intravenous phosphate load. The patient was started on a diet which contained approximately 1,200 mg. of phosphorus and 1,280 mg. of calcium per day. His daily urinary phosphate excretion ranged from 690 mg. to 765 mg. per day.

He responded to three intramuscular injections of 3 cc. of parathyroid hormone given at eight-hour intervals, by a marked increase in daily phosphate output and a marked lowering of tubular phosphate reabsorption. At the same time the serum calcium level rose to 9.4 mg. and the phosphorus dropped to 5.8 mg. per 100 cc. He was then given 100,000 units of vitamin D daily by mouth. His phosphate clearances and daily phosphate output reverted to control levels.

While receiving 100,000 units of vitamin D per day the serum phosphorus level remained at approximately 7 mg. and the calcium ranged from 7.9 to 8.6 mg. per 100 cc. He was discharged on this regimen, and ten days later the serum phosphorus level was still 7 mg. per 100 cc., but the calcium had risen to 9.5 mg. At this time his vitamin D intake was raised to 200,000 units daily. A month later his conjunctivitis and photophobia had disappeared, his toenails were clearer at the bases, but there was no growth of hair. The concentration of serum phosphorus was 7.4 mg. and of calcium, 11.6 mg. per 100 cc.

The patient was next seen a month later, at which time it was noted that he had polyuria, anorexia, and injection of the lateral aspects of each eye. He had lost weight and was said to tire easily. It was felt that he was showing signs of vitamin D toxicity, and his intake of vitamin D was cut to 50,000 units per day. At this time the serum calcium concentration was 13.7 mg. and the serum phosphorus, 5.2 mg. per 100 cc. On repetition, a phosphate clearance study showed a marked decrease in phosphate reabsorption and an overall increase in excretion, although the inulin clearance had fallen from a previous average of about 85 cc. per minute to 30 cc. per minute. Vitamin D administration was

discontinued. His urinary excretion of 17-ketosteroids was 2.03 mg. per twenty-four hours, which is in the low normal range for this laboratory. Assay for urinary gonadotropins gave negative results at 6 mouse units.

Nine days later he was readmitted because of vomiting. Physical examination showed marked dehydration, lassitude and a diffuse increase in pigmentation. Serum sodium concentration was 134 mEq., chloride 96 mEq., potassium 3.7 mEq., and CO_2 23 mEq./L.; calcium 14.1 mg. per 100 cc., phosphorus 5.4 mg. and alkaline phosphatase 3.75 Bodansky units per 100 cc. The Sulkowitch test showed a moderate amount of calcium in the urine.

At this time a diagnosis of Addison's disease was considered because of the pigmentation and the clinical picture. However, the urinary ketosteroid value obtained during the preceding week, and the blood chemical values were thought to be against this diagnosis, as was his rapid recovery after the infusion of 1 liter of 5 per cent glucose containing only 100 cc. of physiologic salt solution.

Ten days later, he was readmitted because he had begun to vomit again. He was weak and had spent most of the preceding seven days in bed, but he had no specific complaints.

Physical examination revealed moderate dehydration, a weight loss of 1.4 Kg. and greatly increased pigmentation, most marked in the skin folds and in the mucosa. The blood pressure at this time was 90/60 and the serum sodium level was 135 mEq., chloride 96 mEq., potassium 4.2 mEq., and CO_2 17.9 mEq./L.

The eosinophil count on the morning of the second hospital day was 337 per cu. mm. Four hours after the administration of 25 mg. of ACTH intramuscularly, the eosinophil level was 213 per cu. mm. Next day, four hours after the last of four doses of 25 mg. of ACTH, the eosinophil count was 500 per cu. mm.

During this time it was obvious that he was becoming progressively dehydrated, as his intake consisted only of sips of ginger ale. His pulse became difficult to obtain and his blood pressure began to fall. The serum sodium level dropped to 129 mEq./L.; the serum chloride was 93 mEq./L., and potassium 4.1 mEq./L. He was given an infusion of physiologic salt solution and 5 per cent glucose with dramatic results. His blood pressure rose to the previous levels, his pulse became normal and he began taking fluids well by mouth. A review of previous x-ray films of the chest was made. His heart had been of normal size until the x-ray examination of ten days before, which had showed microcardia. His heart size returned to normal following the infusion of salt and glucose.

The diagnosis of Addison's disease was thus established and he was treated with cortisone by mouth and the addition of salt to his diet. He responded well, gaining 1.8 Kg. during the twenty days of his hospital stay.

Figure 1 graphically portrays the laboratory findings before and after the diagnosis of Addison's disease was made. The ketosteroid excretion seventeen days before the last admission was 2.03 mg. per twenty-four hours, and on the day of admission was 0.4 mg. There was no rise with ACTH. The excretion of 17,21,dihydroxy,20-ketosteroids measured by the method of Gornall (4), which had formerly been 0.8 mg. per twenty-four hours, was 0.2 mg. on admission and 0.4 mg. for the day of ACTH administration. With the administration of cortisone the ketosteroids rose to a high of 7.9 mg. per twenty-four hours and 17,21dihydroxy,20-ketosteroid excretion was 1.8 mg. However, the androgen excretion (bioassay) did not change and was about equal to 100 gamma of testosterone per day, both at the time of the previous admission and before and after treatment with cortisone and ACTH.

Serum potassium levels are not given in Figure 1, as all values were between 3.5 and

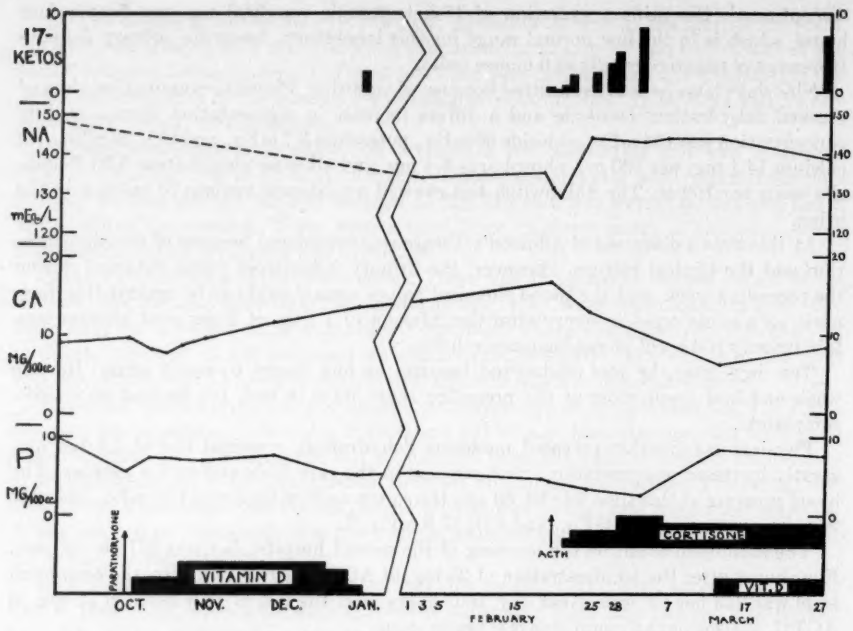

FIG. 1. Urinary ketosteroid excretion and serum sodium, calcium and phosphorus levels over a six-month period, during various forms of therapy.

4.6 mEq./L. Only a single sodium level (148 mEq./L.) was recorded for the first three months of observation. The next sodium determination, made at the time of the first admission for vomiting and weakness, was 134 mEq./L. On the last admission, the serum sodium level was 135 mEq./L. and fell to 131 mEq./L. before ACTH was given; at the end of the twenty-four hours of ACTH it was 129 mEq./L. With cortisone administration and the addition of 2.5 grams of salt to the diet, the serum sodium concentration rose to 144 mEq./L. and thereafter remained normal, as did the serum potassium concentration, in spite of changing cortisone dosage in the four months after discharge. Fasting blood sugar values were always within normal limits.

As may be seen from Figure 1 the serum phosphorus level fell markedly and rapidly when parathyroid hormone was administered, only to rise to a level of 7 mg. per 100 cc. and remain there for two months, while the patient received 100,000 units of vitamin D daily. When signs of vitamin D toxicity began to appear the serum phosphorus value dropped to 5.5 mg. per 100 cc., where it remained for the next month, although vitamin D administration was discontinued. While in the hospital during the last admission, the phosphorus level dropped to 2.5 mg. per 100 cc., only to rise after discharge. The concentration of serum calcium rose with the administration of parathyroid hormone and then dropped and remained at about 8 mg. per 100 cc. during the next month of vitamin D administration. It then gradually rose to the toxic level of 14 mg.; it was reported as 17 mg. when the patient was acutely dehydrated in addisonian crisis; four days thereafter, it was back to 13 mg. and it had fallen to 6.4 mg. one week after dis-

charge from the hospital (last admission). Within the next two weeks, with the administration of 100,000 units of vitamin D per day, it rose to 8 mg. per 100 cc. At this time the dosage of cortisone was lowered to 50 mg. per day. One month later, without further change in therapy, the serum calcium level was 11 mg., and phosphorus 6.4 mg. per 100 cc. At this time the cortisone dosage was further reduced to 25 mg. per day and the vitamin D dosage was continued at 100,000 units per day. One month later the patient was seen again; he had anorexia, polyuria, an increase in pigmentation and was easily fatigued; he had lost 1.5 Kg. It seemed obvious that his Addison's disease was not well controlled and the dosage of cortisone was once more raised to 50 mg. per day. The serum calcium level was 13.6 mg. at this time and the phosphorus, 5.25 mg. per 100 cc.. Vitamin D was continued without change at 100,000 units per day by mouth. His symptoms of weakness, fatigue and anorexia disappeared within a week. Polyuria disappeared at this time also, but returned two weeks later. The boy was seen three weeks after the previous visit. He had regained the weight he had lost and was clinically well. He was so well tanned that it was impossible to judge his pigmentation. At this time the serum calcium level was 11.4 mg. and the phosphorus, 4.6 mg. per 100 cc. Because of the polyuria, and in spite of the absence of any other noticeable abnormalities, vitamin D dosage was reduced to 50,000 units per day. The polyuria disappeared in four days.

DISCUSSION

The diagnosis of hypoparathyroidism was easily established in this boy by the clinical and laboratory findings as well as by the response to parathyroid extract. Although he showed no rise in the level of serum potassium, the diagnosis of renal salt-wasting syndrome instead of Addison's disease need not be considered, because of the pigmentation and steroid and eosinophil findings before and after ACTH and because of his clinical relapse when cortisone was cut below an adequate level.

It is more difficult, however, to establish the exact time of onset of the adrenal insufficiency. The first finding indicative of this condition may have been the lowered glomerular filtration rate noticed one week before the first obvious clinical findings of adrenal insufficiency. However, since the patient had signs of vitamin D intoxication at the time, this is open to question. One week later increased pigmentation of the addisonian type was easily demonstrable.

Just as it is impossible to say when the Addison's disease began, it is also impossible to state categorically that the adrenal insufficiency had an ameliorating effect on the symptoms of the hypoparathyroidism. There are several factors that suggest this effect. Figure 2 shows the changes in phosphate excretion during the administration of cortisone. After ten days of such therapy, the phosphate excretion had dropped to 112 mg. per day compared to the 500 or 600 mg. per day the patient had been excreting before cortisone therapy was begun, although the intake of phosphate actually rose from zero to at least 1 gram per day. The decreased output cannot be explained by the drop in serum phosphorus concentration, since the ex-

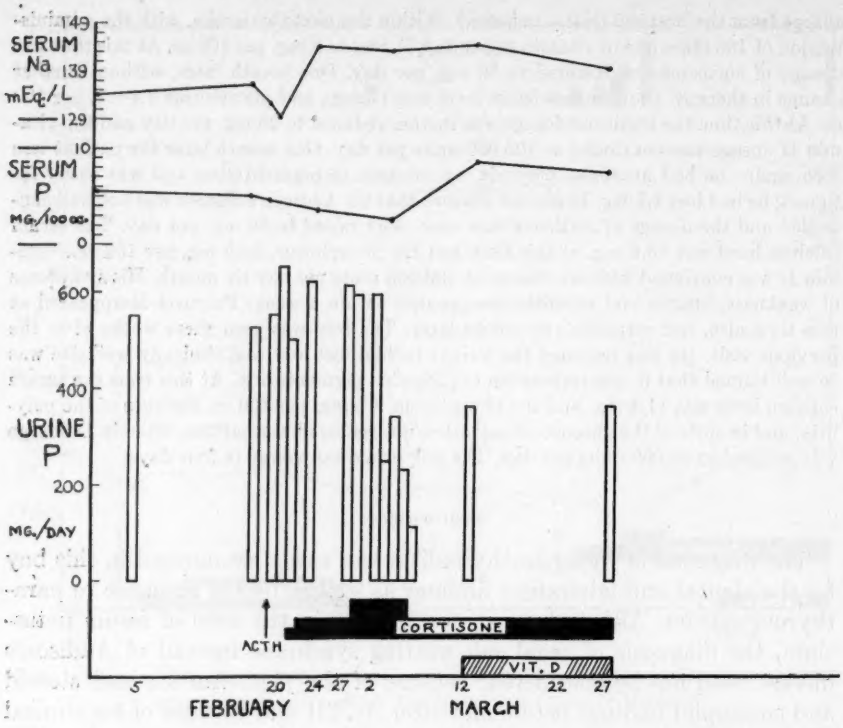

FIG. 2. Phosphate excretion before and after control of Addison's disease with cortisone and sodium chloride.

creted phosphate is a much smaller percentage of the phosphate filtered, when the amount filtered is estimated on the basis of daily creatinine excretion. The patient had had no vitamin D for one month but, because of the previous administration, there can be no certainty that the increased phosphate excretion prior to cortisone therapy was due to adrenal insufficiency. Nevertheless, it is a possible interpretation. This possibility is also suggested by the case reported by Sutphin, Albright and McCune (3). Their patient had tetany with low serum calcium and phosphorus levels at the time he was first seen by them because of the onset of Addison's disease. McQuarrie, Ziegler and Hansen reported a child with idiopathic hypoparathyroidism (5). This boy was followed for several years and then signs and symptoms of Addison's disease developed. Dr. Hansen found that the patient's Chvostek and Trousseau signs had disappeared coincident with the development of adrenal insufficiency (6). At postmortem examination there was no evidence of parathyroid tissue and practically no trace of the adre-

nals. Vitamin D had not been noted to decrease the tubular reabsorption of phosphate in other cases of hypoparathyroidism (7), and it has been noted at times to increase the glomerular filtration rate; it also has non-renal effects. However, it is possible that the effect in our patient may have been a toxic one. Finally, the development of high calcium and lowered phosphorus levels in serum when cortisone dosage was decreased during constant vitamin D dosage, and then the disappearance of these findings when cortisone administration was increased, bear out this thesis. On the other hand, the administration of cortisone to animals without hypoparathyroidism apparently has the opposite effect (8). Increased phosphate excretion has been seen in normal children given ACTH, and in a boy with Addison's disease given cortisone. This change was produced by an increase in the filtration rate of phosphate (9).

The etiology of this syndrome is still in doubt. It is possible that vitamin D intoxication could lead to destruction of the adrenals and produce addisonian symptoms. In this patient, however, there were no signs of calcification in the adrenal region. The toxicity was of a short duration and cleared up promptly with cessation of vitamin D administration. In the 2 cases in the literature in which there was a postmortem examination, there was atrophy of the adrenals but there were no signs of calcific deposits. Furthermore hypoparathyroidism and Addison's disease have co-existed in patients in whom the symptoms of Addison's disease preceded those of hypoparathyroidism (2). The second possibility is the production of this syndrome by the destruction of both parathyroids and adrenals by a specific infection such as moniliasis or tuberculosis. However, repeated tuberculin and histoplasmin skin tests gave negative results in this patient; and monilia could not be cultured from the nose, throat and nails, although several attempts were made. Furthermore, there was no suggestion of such infection in either of the 2 autopsy cases reported in the literature.

Dr. Josef Warkany has permitted the authors to quote from his experience (10). He has observed a patient with Addison's disease whose sister has hypoparathyroidism. These 2 patients, coupled with the patient described here and the other similar cases, have led him to suggest that the coexistence of Addison's disease and hypoparathyroidism may be a variable expression of the same hereditary trait. In any event, the disease in this patient was not thought to be due to vitamin D toxicity or to any specific infection.

SUMMARY

The case is reported of a 10-year-old boy who had hypoparathyroidism for four and a half years before Addison's disease developed. Evidence is presented which suggests that the adrenal insufficiency had an ameliorating

effect on the hypoparathyroidism. It was felt that the syndrome in this case was due neither to a specific infection nor to vitamin D intoxication. The possibility of an hereditary trait has been considered.

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EFFECT OF DESOXYCORTICOSTERONE ACETATE UPON THE BLOOD PRESSURE OF RATS FED VARIED DIETARY INTAKES OF POTASSIUM AND SODIUM*

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IN A previous communication (1) from this laboratory it was shown that adequate potassium intake is requisite for the development and persistence of hypertension following administration of desoxycorticosterone acetate (DCA) to intact rats. This study suggested the importance of determining the interrelationship of sodium and potassium ions in DCA-induced hypertension and whether or not excessive potassium intake potentiated the pressor effect of DCA. In order to clarify these problems, the present investigation was undertaken.

METHODS

In approximately one half of a series of rats subjected by us to uninephrectomy and the application of a figure-of-eight ligature to the remaining kidney, hypertension failed to develop postoperatively. It was found previously (2) that the administration of DCA rapidly elevated the blood pressures of such normotensive postoperative rats. This method was utilized in the present study to induce hypertension.

A series of five-week-old male rats (Long-Evans) was uninephrectomized and a renal ligature applied to the remaining kidney (3). Blood pressures were obtained at weekly intervals postoperatively with the microphonic manometer (4). By the ninth week 48 rats that failed to show systolic blood pressures exceeding 135 mm. of Hg were divided into five groups which thereafter were maintained upon synthetic diets containing varied amounts of sodium and potassium as indicated in the table on page 662. The basic potassium-deficient diet fed to Groups III and V has been described previously (5). During the subsequent ten-week interval all rats

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Group	No. of rats	Dietary content (% of diet)		Drinking fluid
		Sodium	Potassium	
I	10	0.7	1.5	Tap water
II	8	0.7	1.5	1% KCl
III	9	0.4	0.006	Tap water
IV	11	0.7	1.5	1% NaCl
V	10	0.4	0.006	1% NaCl

received tri-weekly subcutaneous injections of 4 mg. of DCA (in benzyl alcohol) and their blood pressures were determined at weekly intervals.

RESULTS

The course of the average weekly blood pressures is depicted in Figure 1. As observed previously (2), all groups of these postoperative but normotensive rats responded to the administration of DCA during the first week with an elevation of the average blood pressures. By the third week, however, there was a divergence in the pressure curves obtained from the various groups. Thus the animals fed a diet with a normal content of sodium and an increased content of potassium (Group I), as well as those fed the same ration but supplemented with 1 per cent KCl solution to drink, continued to show further elevations of blood pressure, which rose to averages of 204 and 194 mm. of Hg. respectively, at the end of the eighth to ninth week. In general, the blood pressure curves were similar in these two groups. The animals of Group III ingested a diet with an adequate content of sodium but a markedly deficient content of potassium. Their average blood pressures reached a peak of 140 to 150 mm. of Hg early in the experimental period, but failed to rise further. Rats of Group IV ingested a diet containing the same amount of potassium and sodium as the diet in Group I, but supplemented with 1 per cent NaCl solution to drink; their blood pressures exhibited a sharp fall in the sixth week, the average pressure declining from 154 mm to 116 mm. of Hg during the subsequent two weeks. The rats of Group V, ingesting the diet markedly deficient in potassium but excessive in its sodium content, had a moderate rise of blood pressure; their average pressure persisted around 135 mm. of Hg until the sixth week, at which time the average pressure abruptly started to fall, and decreased from 133 mm. to 92 mm. of Hg during the next two weeks.

No outward evidence of toxicity was observed in the rats receiving diets with an adequate or excessive potassium content and a normal sodium content (Groups I and II). On the other hand, subnormal weight gain or loss of weight occurred in the rats ingesting a diet containing excess sodium

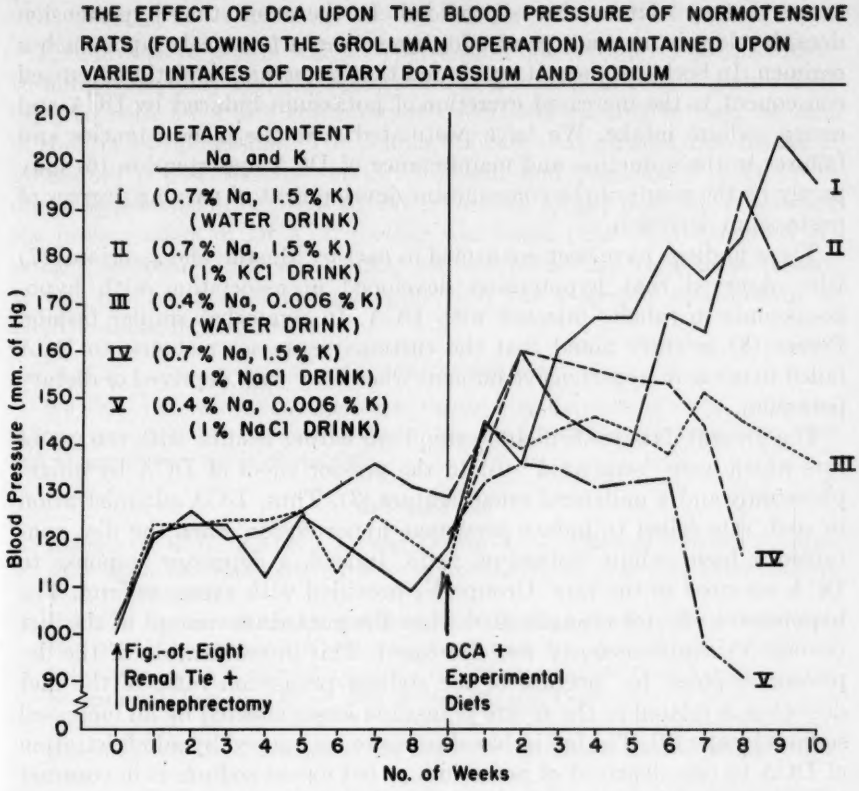
THE EFFECT OF DCA UPON THE BLOOD PRESSURE OF NORMOTENSIVE RATS (FOLLOWING THE GROLLMAN OPERATION) MAINTAINED UPON VARIED INTAKES OF DIETARY POTASSIUM AND SODIUM
DIETARY CONTENT
Na and K
I (0.7% Na, 1.5% K) (WATER DRINK)
II (0.7% Na, 1.5% K) (1% KCl DRINK)
III (0.4% Na, 0.006% K) (WATER DRINK)
IV (0.7% Na, 1.5% K) (1% NaCl DRINK)
V (0.4% Na, 0.006% K) (1% NaCl DRINK)
Blood Pressure (mm. of Hg)
210
200
190
180
170
160
150
140
130
120
110
100
90
0
Fig.-of-Eight Renal Tie + Uninephrectomy
DCA + Experimental Diets
0 1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 10
No. of Weeks

FIGURE 1

(Groups IV and V) or a diet which was deficient in potassium (Group III); and about half of the animals of each group died during the final week of the experimental interval. Blood pressure values of obviously sick rats were not included in the results.

DISCUSSION

We have demonstrated previously (1) that administration of DCA to rats fed a diet deficient in the cation, potassium, failed to induce an elevation of blood pressure. Actually a depressor response to DCA administration was obtained under these conditions, particularly if excess dietary sodium intake was provided. Moreover, it was observed that even when intact rats ingest a ration containing a normal content of potassium, hypertension frequently fails to develop following administration of DCA, if

excess dietary sodium also is provided. In the event that hypertension does develop, it may not persist for more than a few weeks upon such a regimen. In both instances it was found that potassium depletion occurred consequent to the increased excretion of potassium induced by DCA and excess sodium intake. We have postulated that the inconsistencies and failures in the induction and maintenance of DCA hypertension (6) may partly be the result of the concomitant development of varying degrees of postassium depletion.

These findings have been confirmed in part by Masson and associates (7) who observed that hypotension developed in association with hypopotassemia in rabbits injected with DCA. In somewhat similar fashion Perera (8) recently noted that the customary pressor response to DCA failed to occur in hypertensive humans when they were deprived of dietary potassiun.

The present data confirm and extend our earlier results, with the use of rats which were "sensitized" (6) to the pressor effect of DCA by uninephrectomy and a unilateral renal ligature (3). Thus, DCA administration in such rats failed to induce persistent hypertension when the diet contained a high sodium/potassium ratio. Indeed, a depressor response to DCA occurred in the rats (Group IV) provided with excess sodium. The hypotensive effect was augmented when the potassium content of the diet (Group V) simultaneously was decreased. This intensification of the depressor response by increase of the sodium/potassium ratio of the diet doubtless is related to the severe potassium losses effected by an increased sodium intake (9). The fall in blood pressure occasioned by administration of DCA to rats deprived of potassium or fed excess sodium is in contrast to the marked hypertension which developed in the rats fed adequate potassium without excess sodium intake. On the other hand, it is significant that further increasing the dietary potassium intake did not appear to augment the pressor effect of DCA.

Apparently the action of the potassium supplement in permitting DCA hypertension to develop is one of replacing the loss of this cation induced by DCA and thus preventing the potassium depletion which would otherwise block its potential pressor effect.

The pattern of general toxic manifestations induced by DCA administration in our rats confirmed previous observations (10) that an increased sodium/potassium ratio in the diet increases the toxic effects of DCA, and that an adequate intake of dietary potassium protects against such toxic effects (11).

SUMMARY

Five groups of rats in which hypertension failed to develop consequent to uninephrectomy and the application of a unilateral renal ligature were injected with DCA and concomitantly fed diets varying in their content of sodium and potassium. The results indicate that an adequate intake of potassium is essential for the development and persistence of DCA-induced hypertension but that excess potassium intake does not potentiate the pressor effect of DCA. It further was found that DCA induces a *depressor* response when the dietary sodium/potassium ratio is increased. It was concluded that the potassium ion has a specific relationship to DCA-induced hypertension.

Acknowledgment

We wish to express our appreciation to the Schering Corporation for the DCA used in these studies, and to Hoffman-La Roche, Inc., for Litrison, used to provide the vitamin supplements of the synthetic diets.

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ALUMINUM HYDROXIDE AS AN ADSORBING AGENT FOR URINARY GONADOTROPINS

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THE concentration of urinary gonadotropins in normal individuals is so low that concentrated extracts must be prepared as free from toxic material as possible with a minimum loss of hormone activity. This laboratory has used the alcohol-precipitation method of Zondek (1) as later modified by Klinefelter *et al.* (2) for determining the urinary gonadotropic substances in male patients with spinal cord injuries. Because of the low gonadotropin levels encountered, the dialysis method was used and it was possible to test for an excretion of less than 6.6 mouse uterine units (m.u.u.) in twenty-four hours with a minimum of toxic reactions in the test animals. Patients on this service were maintained on a well hydrated regimen; consequently, daily urine volumes would be as much as 8 liters. Because of the large urine volumes obtained, adsorption concentration techniques were examined. Katzman *et al.* (3) demonstrated that chorionic gonadotropins could be adsorbed on a permutit column and later both Dekanski (4) and Bradbury *et al.* (5), working independently, described adsorption methods with the use of "kaolin" in the recovery of chorionic and pituitary gonadotropins from urine.

These adsorption methods have three chief advantages: 1) they are less time-consuming and, therefore, premit frequent determinations; 2) they do not require the use of large volumes of alcohol and large pieces of laboratory equipment; and 3) they are free from detoxifying procedures that would result in a loss of hormone activity. From the data reported the results are comparable to those with the alcohol-precipitation method.

One of the chief difficulties of the alcohol-precipitation method is presented by a large urine volume. The paraplegic patients in this study had 24-hour urine volumes that varied between 3 and 8 liters. The alcohol-precipitation method required four times that volume of alcohol; this resulted in very tedious and time-consuming separation procedures and consequent limitation in the number of determinations.

A few kaolin adsorption determinations were tried with the method of

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Dekanski (4) and the results were comparable to those obtained with alcohol-precipitation. However, it was difficult to obtain a good separation by centrifugation; although settling and decanting worked well, this procedure consumed as much time as the precipitation with alcohol.

Aluminum hydroxide was then tried as an adsorbing agent in place of kaolin. It was found to remove gonadotropic substances from the urine at pH 4.0 and these substances could be eluted at pH 10. Aluminum hydroxide was insoluble at a wide range of PH and seemed to offer two advantages as an adsorbing agent over the use of kaolin. 1) Kaolin clay ($H_2Al_2(SiO_4)_2 \cdot H_2O$) is a natural product resulting from the weathering and action of water and carbon dioxide on pure feldspar ($KAlSi_3O_8$) and must be purified before use. It is probable that different batches of purified kaolin will not have the same impurities and will not display the same adsorptive properties. 2) Aluminum hydroxide can be separated from suspension by centrifugation more readily than kaolin. Aluminum hydroxide is a material that is easily prepared in the laboratory from standard reagents and no further treatment is necessary other than washing with water.

The alcohol-precipitation-dialysis method, as previously mentioned, was compared with the aluminum hydroxide adsorption method for quantitative recovery and duplication of results. The comparison tests consisted of four groups. 1) Urinary gonadotropins were determined on 24-hour urine specimens collected from 8 normal male subjects, using both the precipitation and adsorption methods on equal aliquots of the specimens. 2) Urine specimens (24-hour) were collected from 2 normal female subjects and concentration was carried out by precipitation and adsorption. 3) Twenty-four-hour urine specimens were collected from 3 female subjects in the first trimester of pregnancy; excess hormone was demonstrated by the Friedman modification of the Aschheim-Zondek test for pregnancy (6). Determinations were made by both methods on equal aliquots of urine in order to demonstrate recovery of high gonadotropic activity. 4) Thirty-six-hour urine specimens were collected from 3 male subjects with known low titers of gonadotropic substances. Pituitary gonadotropin of equine origin, previously assayed in terms of rat ovarian units, was added to the native urine in predetermined doses and both methods of concentration were used, to test recovery.

MATERIALS AND METHODS

Alcohol-precipitation-dialysis method. The urine was precipitated with four volumes of ethyl alcohol and the precipitate was collected and dried. The dry material was suspended in water and transferred to a cellophane bag and dialyzed against tap water for four hours. The clear dialysate was precipitated with four volumes of alcohol and the precipitate again collected and dried. When ready for assay, the precipitate was extracted

with water and suitable dilutions for assay were prepared.

Aluminum hydroxide adsorption method. The aluminum hydroxide suspension was prepared by mixing equal volumes of 2N sodium hydroxide and 2N aluminum sulfate:

$$Al_2(SO_4)_3 + 6NaOH \rightarrow 2Al(OH)_3 + 3Na_2SO_4$$

The aluminum hydroxide was washed with water by decantation after centrifugation until free of excess reagents and a 10 per cent suspension was prepared. The suspension was adjusted to pH 4.0 with 20 per cent hydrochloric acid.

1. A 12-hour or 16-hour aliquot of urine was separated from the well-mixed and filtered specimen. This was adjusted to pH 4.0 with 10 per cent hydrochloric acid.

2. To the urine was added 5 volumes per cent of the 10 per cent aluminum hydroxide suspension and the specimen shaken by hand for five minutes.

3. The aluminum hydroxide was separated by centrifugation and the supernatant solution was discarded. The aluminum hydroxide was washed once with 0.1N acetic acid, using approximately twice the volume of the precipitate; this step replaced the dialysis. Again the aluminum hydroxide was separated by centrifugation.

4. The gonadotropins were eluted from the aluminum hydroxide by adding normal sodium hydroxide until the pH approached 10, the final adjustment to pH 10 being made with 0.1N sodium hydroxide. The precipitate was centrifuged and the supernatant liquid saved.

5. Tenth normal hydrochloric acid was added dropwise to the sodium hydroxide solution until pH 5.5–6.0 was reached.

6. The proteins containing the gonadotropic substances were precipitated with five volumes of cold acetone.

7. The precipitate was separated by centrifugation, washed once with ether, and dried under slight vacuum. The extract was stored in the refrigerator until ready for bioassay.

8. The precipitate was extracted with 11 cc. of water if a 16-hour aliquot was concentrated, or with 8.25 cc. if a 12-hour aliquot was used.

Assay. The assay method of Levin and Tyndale (7) as modified by Klinefelter *et al.* (2) was used, with one modification concerning the criteria of a positive response. By definition, the result of a test was considered positive if the uterus of the test animal weighed at least 100 per cent more than that of a similarly injected control mouse.

1. Two 21-day-old female white mice weighing from 7 to 10 grams were used at each dilution level. They were injected subcutaneously five times over a period of three days (twice the first day, twice the second and once the third day) with 0.5 cc. of extract each time. The animals were sacrificed with chloroform seventy-two hours after the first injection. The uterus was recovered, blotted dry and weighed on a precision balance to 0.1 mg.

2. At least 2 animals were used as negative controls; *i.e.*, they were similarly injected with physiologic sodium chloride, and the uterine weights recorded. Postive controls were also used; *i.e.*, animals were injected with pituitary gonadotropins and their uterine weights determined.

Calculations. Since a mouse will tolerate only 2.5 cc. of fluid divided as described, one can, by injecting untreated urine, test for a minimum of 40 mouse uterine units (m.u.u.) per 100 cc. of urine. With the use of precipitation methods combined with dialysis, the hormone can be concentrated into a small volume.

The final solutions for injections (8.25 cc. or 11 cc.) represented the entire 12-hour or 16-hour urine collection. If 2.5 cc. gave a positive result the entire specimen contained

at least 3.3 or 4.4 m.u.u. of gonadotropins per twelve or sixteen hours, respectively; the minimum amount per twenty-four hours was easily calculated.

To test for increased amounts of gonadotropins, dilutions of 1:1 were made with distilled water. The tests were repeated until a dilution was reached at which there was not a positive (100 per cent increase) uterine weight. It was important to treat the mice exactly alike in respect to volume of injection, time of injection, and degree of maintenance care.

RESULTS AND DISCUSSION

It was shown that the adsorption procedure eliminated large volumes that had made the steps of precipitation, separation and dialysis very cumbersome. Aluminum hydroxide adsorption procedures can be handled entirely by shaking, decantation, and centrifugation at moderate speeds. The reagents and equipment are both inexpensive and commonly available in the laboratory.

Table IA shows the results in 8 normal men. The values by both methods were comparable at reasonable dilutions, and were in agreement with normal values reported in the literature, *viz.*, more than 6 and less than 53 mouse units. Table 1B shows the results in 2 normal women. Subject S.R. showed slightly higher values, and Subject M.M. showed slightly lower

TABLE 1. GONADOTROPIN EXCRETION: COMPARISON OF METHODS

Subject	Aluminum hydroxide adsorption method		Alcohol precipitation method	
	More than (m.u.u.)	Less than (m.u.u.)	More than (m.u.u.)	Less than (m.u.u.)
	A. *Normal males*			
J.G.	13.2	26.4	13.2	26.4
R.M.	6.6	13.2	6.6	13.2
J.D.	26.4	52.8	26.4	52.8
J.V.	6.6	13.2	6.6	13.2
D.H.	6.6	13.2	6.6	13.2
A.B.	26.4	52.8	26.4	52.8
M.A.	26.4	52.8	26.4	52.8
A.C.	13.2	26.4	13.2	26.4
	B. *Normal females*			
S.R.	52.8	105.6	52.8	105.6
M.M.	6.6	13.2	6.6	13.2
	C. *Pregnant females*			
R.S.	976	1952	976	1952
X.M.	1952	3904	1952	3904
T.M.	3904	4888	3904	4888

TABLE 2. RECOVERY OF PITUITARY GONADOTROPINS BY ADSORPTION AS COMPARED WITH PRECIPITATION

Specimen No.	Urine vol. (cc.)	Rat units of pit. gon. added	Mouse uterine units recovered			
			Adsorption		Precipitation	
			More than	Less than	More than	Less than
1	700	0		6.6		6.6
2	800	0		6.6		6.6
3	700	0		6.6		6.6
4	700	5	7.9	10.5	7.9	10.5
5	700	10	13.2	17.5	13.2	17.5
6	700	20	17.0	28.4	17.0	28.4
7	700	30	44.0	52.8	44.0	52.8
8	700	50	52.8	88	52.8	88
9	700	100	120	176	120	176
10	700	150	145	240	145	240
11	700	200	334	400	334	400

values than are usually reported. In both cases, however, the two methods recovered comparable amounts of hormone. Table 1C shows recovery of high concentrations of chorionic gonadotropin by both methods in pregnant females. The dilution response was identical, with either method. Table 2 shows the quantitative recovery of pituitary gonadotropins, from low to relatively high concentrations. In this assay, rat ovarian units of pituitary gonadotropin were added to the specimen, and mouse uterine units were reported in the assay. From the data it appears that 1 rat ovarian unit was equivalent to 1.5 mouse uterine units. Both methods showed identical recovery over the range of dilutions used. It may be seen that the aluminum hydroxide procedure gave comparable values, in both low and high concentrations. The range of the method makes it applicable for patients with increased gonadotropin excretion. Urinary gonadotropin determinations with the alcohol-precipitation and aluminum-hydroxide-adsorption methods were made on 16 patients with traumatic spinal cord lesions, at least three separate times over a period of nine months. In all cases the results could be duplicated by either method. These cases are not included in the tables; with few exceptions they showed less than 6.6 mouse units of gonadotropins per twenty-four hours.

SUMMARY

A method is described for adsorbing urinary gonadotropin with aluminum hydroxide. The method is found to be an efficient, simple and quick

procedure, the nontoxic concentrates being suitable for further concentration and purification. Hormone assays carried out by this method are comparable in sensitivity with the usual alcohol-precipitation method but are more rapid and less expensive.

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Letters to the Editor

METHOD FOR PREOPERATIVE DIFFERENTIATION BETWEEN THE BENIGN AND THE POSSIBLY MALIGNANT SOLITARY NONTOXIC THYROID NODULE*

To the Editor:

It is at present widely accepted that a significant percentage of solitary nontoxic thyroid nodules are histologically malignant. Surgical removal has therefore become the therapy of choice for solitary nodules. It is also true that thyroid surgery, in all but the most expert hands, is attended by a significant number of operative complications and a definite, though small, mortality rate. If all patients with solitary nontoxic nodules are operated upon in order to rule out malignancy, then approximately 80 per cent will have had needless surgery, since in most instances no other indication for surgery exists.

A preoperative laboratory test which would differentiate between the benign and the malignant nontoxic nodules would thus be of value to the clinician in avoiding unnecessary surgery. Dobyns, Skanse and Maloof (1), while estimating the function of thyroid nodules, noted that carcinomatous thyroid tissue collected less radioiodine (I^{131}) than did normal thyroid tissue. Means (2) later reported that "so far, we have never found a 'hot' nodule which has shown histologic evidence of malignancy." Thus it would seem that a diagnostic procedure may be available for differentiating between malignant and benign nodules, based upon their avidity for I^{131} relative to the non-nodular thyroid tissue.

In patients with solitary nodules, after the ingestion of a tracer dose of I^{131}, directional counts over the nodule and the non-nodular tissue were made by means of a lead-encased Geiger counter. The collection of I^{131} by the nodule was compared with the collection by a similar area of the normal part of the thyroid gland. The nodules were then classified as "cold," "warm," or "hot," depending upon whether their respective uptakes were

* This investigation was supported in part by research grant A-169 from the Division of Research Grants, National Institutes of Health, Public Health Service. These studies were made at the Maimonides Hospital of Brooklyn and the Brooklyn Veterans Administration Hospital.

less than, about the same as, or more than that of the non-nodular thyroid tissue. The part of the thyroid gland containing the nodule was then surgically removed.

Our experience thus far with 24 "hot" nodules is that all of them were benign. On the other hand, 20 per cent of the 21 "warm" nodules and 32 per cent of the 40 "cold" nodules were reported to be malignant pathologically. Thus, if more extensive experience confirms the benignancy of "hot" nodules, a simple laboratory method will be available to spare a significant proportion of patients with nontoxic single thyroid nodules from the risks of surgery.

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PITUITARY IRRADIATION FOR BILATERAL PROGRESSIVE EXOPHTHALMOS

TO THE EDITOR:

Recent reports have indicated that x-ray treatment to the pituitary may cause a regression of bilateral progressive exophthalmos (1–8). The causative relation between the treatment used and results achieved is difficult to evaluate because early, fluctuating exophthalmos is frequently self-limited and may subside spontaneously. Moreover, published reports do not clearly indicate whether the effect is the result of irradiation of the pituitary-hypothalamic area or of the orbits.

In an attempt to ascertain the value of this procedure, we subjected 10 patients with malignant exophthalmos (Table 1) to x-ray treatment to the pituitary. The physical factors were: 400 kv, HVL 2.4 mm. Cu, TSD 70–75 cm. One patient was treated with 200 kv generated x-ray, HVL 0.9 mm. Cu, TSD 50 cm. Seven patients received treatment through four fields, 2 patients through six fields, and 1 patient by $2\times180°$ rotational method. The field size varied from 1.5 cm. diameter to 3 cm. diameter. The

radiation beams were centered upon the pituitary, carefully excluding the orbits. Beam localization was secured by a method previously described (9). Treatment was given in one course; 25 to 29 treatments over a period

TABLE 1

	Sex	Age (yrs.)	Diagnosis	Duration of exoph.	Before treatment		After treatment		Duration of follow-up after treatment	Comments
					OS	OD	OS	OD		
1	F	42	Recurrent Graves' dis.	15 mos.	21.0	22.0	21.5	23.0	6 mos.	Concurrently hyperthyroid
2	F	40	Euthyroidism	3 yrs.	19.0	22.0	19.0	22.0	15 mos.	
3	F	32	Post-Graves' dis.	4 yrs.	27.5	29.0	28.0	29.0	5 mos.	
4	M	31	Euthyroidism	8 mos.	23.5	19.0	28.0	21.0	10 mos.	
5	F	31	Post-Graves' dis.	5 yrs. 4 mos.	27.0	27.0	26.5	27.5	14 mos.	
6	F	49	Post-Graves' dis.	8 yrs.	21.5	21.5	21.0	20.5	15 mos.	Localized myxedema (unchanged)
7	F	49	Post-Graves' dis.	4 yrs.	28.0	26.0	27.5	26.5	1 yr.	Zondek's syndrome (10)
8	M	27	Post-Graves' dis.	9 yrs.	28.0	31.0	28.0	29.5	7 mos.	
9	F	57	Post-Graves' dis.	"Many years"	22.5	23.5	23.0	23.0	13 mos.	
10	F	27	Post-Graves' dis.	4½ yrs.	34.0	32.0	35.0	32.0	5 mos.	

of thirty-five to thirty-nine days. The patient who was treated with 200 kv x-rays received 21 treatments in thirty-three days. The treatment dose estimated in the pituitary (tissue dose) was approximately 3500 r.

Patients were selected whose exophthalmos was stable and had not been controlled by administration of thyroid substance, iodine, ammonium chloride, restriction of salt, elevation of the head at night, or by treatment with hyaluronidase, parahydroxypropiophenone or estrogens. In addition to symptoms and physical findings, we recorded exophthalmometric measurements, estimation of retro-orbital tension, and urinary excretion of 17-ketosteroids and pituitary gonadotropins.

Subsequent to therapy, no change was noted in any of these. There was likewise no clinical or laboratory evidence of altered anterior pituitary function.

Our failure to produce a remission of the exophthalmos may be due to either or both of two causes: 1) the long duration of the disease, and 2) the fact that we did not irradiate the orbit. Beierwaltes (8) has recently pointed out that favorable results are most often achieved in patients whose exophthalmos is of short duration. Published reports of improvement in progressive exophthalmos after x-ray therapy do not indicate clearly whether radiation was limited to the area of the pituitary and hypothalamus. Judging from the field sizes used in irradiation by this author (usually 7×7 cm.), the pituitary, together with the retro-orbital tissues and the hypothalamus, were simultaneously exposed to the radiation beam. If the orbit was ir-

radiated, certain areas, especially sites of lymphorrhagic infiltration, might well be more sensitive than older fibrotic areas. In long-standing exophthalmos, irreversible changes may have occurred in the extraocular muscles and retro-orbital tissues. On the other hand, it is difficult to evaluate the effects of treatment in early cases, in which remission may occur without treatment.

In conclusion, our results show no favorable effects of "pituitary" irradiation upon long-standing exophthalmos. They also show that a radiation dose (tissue dose) of around 3500 r delivered in approximately thirty-five days has no harmful effects upon pituitary function, and therefore does not preclude the use of such radiation treatment in progressive exophthalmos of brief duration.

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ISOLATION OF PREGNANE-3-ALPHA,17-ALPHA,21-TRIOL-20-ONE (TETRAHYDRO COMPOUND S) FROM THE URINE OF A WOMAN WITH METASTATIC ADRENOCORTICAL CARCINOMA*

To the Editor:

Pregnane-3-alpha,17-alpha,21-triol-20-one (tetrahydro compound S) has been isolated from the urine of a 48-year-old woman with metastases from a previously removed adrenocortical carcinoma. This case has been previously described in considerable detail (1). The patient showed marked hirsutism, acne, a low pitched voice, an enlarged clitoris, atrophy of the breasts, and marked muscular weakness, associated with abnormalities of electrolyte metabolism at times similar to those found in Cushing's syndrome, but she had periods of *hypo*glycemia (prior to, and after development of recognizable liver metastases). The urine for chromatography was collected one month before her death. At approximately this time, the total neutral 17-ketosteroid excretion varied between 162 and 320 mg. per twenty-four hours. Dehydroisoandrosterone excretion was 27 mg. (method of Allen *et al.* (2)) and urinary estrogens were greater than 400 and less than 660 mouse units per twenty-four hours. Neutral lipid-soluble reducing substances found in the extract subjected to paper chromatography totaled 64 mg. per twenty-four hours (high normal by the method used is less than 20 mg.). A semiquantitative estimate indicated that the 24-hour urine collection contained approximately 12 mg. of tetrahydro compound S (extractable by the method employed).

The urine was adjusted to pH 4.5 with acetic acid and sodium acetate and incubated for forty-eight hours with beta-glucuronidase (100 units per cc.). After acidification to pH 1 with hydrochloric acid, the urine was extracted with equal volumes of chloroform on an oscillating shaker for one hour. Paper chromatography was performed by the method of Burton, Zaffaroni and Keutmann (3). Chromatography, using 1.1 per cent of the 24-hour urine extract, showed a large blue tetrazolium-reacting zone in the position where tetrahydro compound S is found. Eluates of this zone from multiple chromatograms run at the same time were examined in various solvent systems and the material was shown to have the same running rates as the sample of pregnane-3-alpha,17-alpha,21-triol-20-one (tetrahydro compound S) kindly supplied by Dr. Thomas F. Gallagher. The material, after acetylation, was further purified by chromatography on silica gel and shown to have absorption spectra in sulfuric acid, according to the method of Zaffaroni (4), duplicating that of the standard. The isolated material was subjected to infrared

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spectrometry through the courtesy of Dr. Thomas F. Gallagher at the Sloan-Kettering Institute for Cancer Research, Memorial Center, New York, and was found to be identical with the standard. There were several other smaller alpha-ketolic zones on the chromatograms. These have not been identified.

Comment: Reichstein and von Euw (5) and others have isolated 17-alpha-hydroxy-11-desoxycorticosterone (compound S) from animal adrenals. More recently, compound S has been identified in blood from the adrenal vein of dogs (6). We have demonstrated that pregnane-3-alpha, 17-alpha, 21-triol-20-one (tetrahydro compound S) is the major alpha-ketolic steroid metabolite of 17-alpha-hydroxy-11-desoxycorticosterone (compound S) found in the urine after administration of this compound to individuals with markedly reduced adrenocortical function (7). It has not been found following the administration of hydrocortisone, cortisone, corticosterone, 11-dehydrocorticosterone, or 11-desoxycorticosterone. It, therefore, seems probable that the adrenocortical tumor tissue secreted the parent compound. The possibility that compound S may be found in the urine is being explored.

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URINARY CORTICOIDS IN PHEOCHROMOCYTOMA

To the Editor:

The view that adrenaline is a physiologic stimulant of adrenocortical secretion by reason of a specific effect on the secretion of ACTH has come under recent question. In animal experiments (1, 2) large doses of adrenaline provoke increased cortical secretion by pituitary stimulation but such stimulation seems to be nonspecific in studies on human subjects (3, 4).

From the clinical aspect, attention has been drawn to the association of adrenocortical adenoma with functioning pheochromocytoma (5) as indirect evidence of adrenocortical stimulation by medullary hypersecretion. On the other hand, with one reported exception (6), patients with functioning pheochromocytoma do not exhibit the clinical signs of hypercorticoidism. A further test of the clinical association between adrenal medullary and cortical functions would be the measurement of urinary corticoids in patients with pheochromocytoma.

In this regard, Sprague and Mason (7) have found increased outputs of "free" formaldehydogenic corticoids (FFC) and 17-ketosteroids in 2 of 14 patients with pheochromocytoma. This report extends these observations by data from 3 adult female patients. The measurements made were FFC (pH 1 extractable) and TFC (pH 1 extractable after glucuronidase hydrolysis), using the methods of Corcoran and Page (8) and of Dustan, Mason and Corcoran, Procedure 1 (9). Diagnoses were made from clinical signs and symptoms, pharmacologic tests, presence of increased urinary catechol amines and demonstration of such in the tumors found at operation, both colorimetrically (method of von Euler and Hellner (10)) and by bioassay.

The data are summarized in Table 1. They show that outputs of FFC were somewhat increased in 1 patient preoperatively, but were normal in 2, whereas in all 3 the outputs of TFC were within or below the normal range.

These observations confirm the impression that adrenocortical hyperfunction is not a significant aspect of pheochromocytoma. The increased outputs of FFC without associated increases in TFC found in one of the subjects recalls the similar pattern found in some patients with essential hypertension (9) and may represent an abnormality of excretion in some patients with hypertensive disease.

The data indicate that prolonged and severe adrenomedullary hyper-

TABLE 1. URINARY FC IN 3 PATIENTS WITH PHEOCHROMOCYTOMA

The days indicated as minus (−) are preoperative; superscript numerals indicate the number of pairs of duplicate analyses for FFC and TFC on pooled specimens, algebraically averaged. Day 0 is the day of operative procedure in each case. During the period 0–4, Patient No. 1 was given 200 mg. of cortisone daily.

Patient No. 1			Patient No. 2			Patient No. 3		
Days	FFC	TFC	Days	FFC	TFC	Days	FFC	TFC
	Mg. per 24 hrs.			Mg. per 24 hrs.			Mg. per 24 hrs.	
-28-0^4	2.15	9.0	-5-0^2	1.0	5.5	-4-0^1	0.47	10.2
0-4^1	6.6	19.7	1^1	1.0	1.9			
5-9^1	3.0	3.7	2-6^2	1.0	10.0			
9-15^1	1.1	1.5						

function does not as such elicit increased activity of the adrenocortical-pituitary axis.

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CORTICOTROPIN-ZINC: CLINICAL OBSERVATIONS ON AN ACTH PREPARATION WITH PROLONGED ACTION

TO THE EDITOR:

A long-acting ACTH preparation (Cortrophine-Z) was tested in humans, the criterion being the decrease in the number of the eosinophils in the blood. This method has the advantage of permitting frequent controls after injection, in order to estimate the duration of the action: this is not so readily accomplished when determinations of 17-ketosteroids and electrolytes are involved.

After a single injection of 20 I.U. of corticotropin-zinc in 22 patients the depression of the eosinophil count was so long maintained that after forty-eight hours the mean value was still lower than the original value. A comparable curve was obtained in another experiment after four injections of 20 I.U. of regular corticotropin at four-hour intervals. The effect of a single injection of 20 I.U. of corticotropin-zinc daily on the 17-ketosteroid, sodium, and potassium excretion was at least as great as that of six injections of 3.5 I.U. of regular corticotropin daily, as indicated by the results of more elaborate tests on 2 patients.

Apart from the foregoing observations, corticotropin-zinc was used in 25 patients with rheumatoid arthritis; the therapeutic effect was essentially the same as the effect obtained by frequent injections of regular ACTH. The daily dose given as a single intramuscular injection never exceeded 20 I.U. of corticotropin-zinc. In some cases, the effect of two injections of 20 I.U. a week was sufficient as a maintenance dose in rather severe forms of rheumatoid arthritis. When using the corticotropin-zinc preparation we did not observe local irritation, allergic reactions, symptoms of resistance (to therapy), or other untoward symptoms. Zinc intoxication would not be expected with the dosage employed here, and no symptoms that could be ascribed to zinc overdosage were observed. Three patients have received this preparation as maintenance therapy for periods ranging from five to seven months.

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CORTICOTROPIN-ZINC: CHEMICAL AND PHARMACOLOGIC INVESTIGATIONS ON A LONG-ACTING ACTH PREPARATION

To the Editor:

Since zinc is known to have a potentiating or/and a retarding effect on the action of certain protein hormones as well as an inhibiting effect on various protein-splitting enzymes, there were obvious reasons to study the influence of zinc salts on the actions of ACTH.

Precipitates of zinc phosphate or zinc hydroxide were produced in aqueous solutions of the hormone at pH values about neutrality.

In this way about 99 per cent of the total activity was precipitated, forming easily injectable suspensions. These preparations showed prolonged and increased activity in a liver glycogen test on hypophysectomized rats (a procedure developed by Organon especially for trial of these products), as well as in the eosinophil test on dogs and in the thymus involution test.

Suspensions for clinical use were prepared *ad hoc* by mixing an acid solution of ACTH (pH ±3) with a buffer solution containing trisodiumphosphate and sodium hydroxide, to yield a suspension of the right composition and pH. The stock solution of ACTH at pH 3 showed excellent stability and retained its ability to form long-acting suspensions even after storage of long duration at elevated temperatures (*e.g.*, six weeks at 45° C.). The ready-for-use mixture with added buffer solution remained active after incubation with serum overnight, in contrast to normal ACTH. This result shows that the ACTH in the zinc complex is protected against destruction by serum enzymes.

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CLINICAL EVALUATION OF CORTICOTROPIN

To the Editor:

When ACTH is administered, the body is stimulated to produce the therapeutically active agent. Satisfactory criteria for the production of active corticoids, and therefore the activity of the administered ACTH, are first, the determination of 17-hydroxycorticoids in blood and, next best, the 24-hour excretion of 17-ketosteroids.

From the results of our clinical investigations the suspicion arose that not all the corticotropin preparations were of the same strength. Using the 24-hour excretion of 17-ketosteroids as a criterion, different batches of corticotropin were tested. From the results of this clinicobiologic test it was concluded that in some batches there was a divergence between the adrenal activation capacity and the activity indicated on the label and based on Sayers' assay.

It is probable that mainly quantitative errors inherent in the Sayers' test can explain these results. However, there also are indications that qualitative differences exist between the ACTH preparations.

For comparative clinical scientific research the use of *one single batch* is therefore obligatory. If this cannot be realized, the effectiveness of the new batch should be compared by the described *clinical* biologic test with the other preparations.

This method has also been successfully applied to the evaluation of long-acting corticotropin preparations, including the new type of ACTH-zinc.

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CYCLICAL INTERMENSTRUAL PAIN: THE MITTELSCHMERZ

TO THE EDITOR:

A report (1) on the occurrence of intermenstrual pain over a period of four and a half years in a married woman (aged 28 at the time of the report) was first made in 1949. The pain occurred regularly except during pregnancy and the first few months of lactation, when it did not occur at all. It seemed clear from the data that the onset of intermenstrual pain was closely related in time to the final stages of ovulation. Records have been carried on continuously since the date of the previous publication and an analysis of the figures obtained for the last five years is now presented.

Table 1 shows the distribution of the duration of the two phases of the menstrual cycle. The mean interval from menstruation to the onset of the pain is 16.3 ± 0.22 days and from the pain to the beginning of menstruation is 13.2 ± 0.10 days. These figures are very similar to those obtained from

TABLE 1. DISTRIBUTION OF DURATION OF PHASES OF MENSTRUAL CYCLE.

Duration of phase (days)	First day of menstruation to Mittelschmerz						Mittelschmerz to onset of menstruation					
	Year						Year					
	1949	1950	1951	1952	1953	Total	1949	1950	1951	1952	1953	Total
12							3	2	2	3	3	13
13							3	5	6	4	6	24
14		2	3		1	6	4	5	5	5	3	22
15	3		4	4	3	14	2					2
16	7	3	2	4	4	20						
17	1	3	2	3	1	10						
18		3	1	1	3	8						
19					1	1						
20												
21		1				1						
22	1		1			2						
	Mean length (± st. error) *16.3±0.22* days											*13.2±0.10* days

the first four and a half years' records (17.3±0.33 and 13.0±0.15) and show once again that the first phase of the cycle is more variable than the second. It is also clear that the variability of the phases has not changed over the last five years.

Table 2 provides information about the side on which the pain was felt. Although the pain was felt more often on the left than on the right side in one year, the predilection for the right side continues (36:25). However, the

TABLE 2. SIDE ON WHICH INTERMENSTRUAL PAIN WAS FELT

	1949	1950	1951	1952	1953	Total
Right	7	5	8	7	9	36
Left	5	7	5	4	4	25

bias is not quite so great as it was previously (29:14). There still seems to be no regularity of alternation from side to side. On one occasion in 1952 there were vague pains on both sides of the pelvis at the expected time of the Mittelschmerz and on another occasion in 1953 the occurrence of two bouts of pain on the same side separated by an interval of a day were reported. These two instances may possibly indicate that two follicles ruptured in each of these cycles.

SUMMARY

The time of onset of intermenstrual pain in a woman (now aged 33) has been recorded for a further five years. The onset of the pain is extremely

regular and occurs 13.2 ± 0.10 days before the beginning of the next menstrual period.

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A CASE OF PITUITARY MYXEDEMA

MYXEDEMA is a rare disorder and its manifestations are protean. For this reason, the underlying disease may go unrecognized because of interest centered upon a particular manifestation or manifestations thereof. The difficulty in diagnosis is compounded when the myxedema is in turn secondary to the even rarer situation, disease of the pituitary—so-called pituitary myxedema. The case report which follows illustrates these several points and brings to attention several potentially fatal complications of pituitary myxedema. There is no clear explanation of why the mucoproteinous deposits of myxedema are only rarely seen in panhypopituitarism in association with levels of serum precipitable iodine frequently as markedly depressed as in primary myxedema. The absence of these deposits might be explained equally well by 1) persistence of some degree of thyroid secretory activity which prevents these changes, or 2) complete inactivity of the entire endocrine system, unlike the usual picture in primary myxedema.

CASE HISTORY

Unit #088124. Second admission (October 13–December 5, 1952). The patient, a 58-year-old business man, was first admitted to the Neurological Institute for the period April 4–10, 1952, because of lethargy, nausea, vomiting, and mental confusion for one and a half weeks. These culminated the morning of admission in a fainting episode in a barber shop. His pulse was noted to have been 54 during the syncopal attack. The family and personal histories were noncontributory, according to the family. This was later confirmed by the patient when he was able to give a history. The previous health and diseases revealed only an episode of a brief delusional episode fifteen years previously. Two months before admission, the patient had had a fall and had injured his right arm; he had also had a "virus infection" with some chilliness for several days two weeks before entry. During the week before entry he had become lethargic, showed a striking change in his usually cheery disposition, and spoke but little.

Physical examination: Blood pressure 110/80, pulse 50, respirations 20, and temperature 98.0° F. The patient was confused and had slight nystagmus (left lateral gaze). The results of examination were recorded as otherwise not remarkable. An admission diagnosis of subdural hematoma or glioblastoma was made.

Laboratory examination: The urine, blood count, hemoglobin and sedimentation rate were normal. The Mazzini test gave a negative reaction for syphilis. A roentgenogram of the skull, and visual field tests showed nothing abnormal. The blood nonprotein

nitrogen level was 20 mg. per 100 ml. and the fasting blood sugar, 97 mg. per 100 ml. Spinal fluid—the cell count, colloidal gold test and Kolmer test showed no deviation from normal; the concentration of total protein was 52 mg. per 100 ml. An electrocardiogram showed low voltage, P-R interval 0.20; ventricular rate 63, and diphasic T-waves throughout; the record was interpreted as abnormal but not characteristic of any particular entity. An electroencephalogram showed an irregular and disorganized record with very low voltage and flattened activity graphs bilaterally; the record was interpreted as showing a mild diffuse abnormality, compatible with subdural hematoma.

The patient was discharged after six days, slightly less confused. The discharge diagnosis was subdural hematoma.

He was not seen again till six months later when he was readmitted to the Neurological Institute for the period October 31–December 5, 1952. Since the first entry, there had been increasing confusion, progressing to a state of intermittent delusions. Changes in appearance, voice, and texture of the skin and hair were denied. There was said to be no intolerance to cold.

Physical examination gave normal findings, except for a pulse of 54.

Laboratory examination: Blood—Hgb 11.8 Gm. per 100 ml.; RBC 3,620,000. Spinal fluid—cell count 3; protein 159 mg. per 100 ml.; and negative reactions with the Kolmer and colloidal gold tests. Ventricular fluid—protein 68 mg. per 100 ml. Serum total protein 5.0 Gm. per 100 ml. (albumin 3.4 Gm., globulin 1.6 Gm.); bromide level normal.

No diagnosis was made. At this point, the patient began to experience a series of syncopal attacks in which he became unresponsive and pulseless. An electrocardiogram showed P-R intervals of from 0.24 to 0.20. Fluoroscopy of the chest showed eventration of the diaphragm with neighboring compression atelectasis of the left lower lobe of the lung, and an uncoiled aorta due to arteriosclerosis. A gastrointestinal x-ray series gave negative results except for the diaphragmatic eventration.

The patient's syncopal episodes continued at intervals of one to several days and became associated with abdominal pain and vomiting. The serum sodium level was 107.8 mEq./L.; after four days of a salt intake totalling 2,400 mEq., it was 107.9 mEq./L. This raised the question of differentiation between salt-wasting of central nervous system origin, adrenal disease, or salt-losing nephritis. Possible adrenal disease could be explained by hypothyroidism, by panhypopituitarism of unknown origin, or by adrenal metastatic involvement from bronchogenic carcinoma. To some observers, the physical examination findings were now strongly suggestive of hypothyroidism, but were not definite enough to exclude the other possibilities.

Further laboratory examination revealed a BMR of −36 per cent and, a 24-hour thyroidal I^{131} uptake of 15 per cent. In a test of the eosinophil response to adrenaline, the count was 113 cells per cu. mm. before injection and 51 cells per cu. mm. three and a half hours after injection, *i.e.*, a fall of 55 per cent. The level of serum cholesterol was 184 mg. per 100 ml.; of potassium, 4.4 mEq./L.; of calculated serum sodium 113.3 mEq./L.; of chlorides, 86.9 mEq./L.; and of CO_2, 17.4 mEq./L.

The patient was given intravenous potassium therapy and for the first time the serum sodium level increased and steadily rose to normal with sustained treatment. No corresponding clinical change ensued. At this point, twenty-seven days after admission, the patient suddenly became semistuporous, and had watery diarrhea, hypotension and a diminished output of urine. He was given whole blood and Lipoadrenal extract. By evening of the same day, the abdomen was tense and distended; no relief resulted from insertion of a nasal tube and administration of prostigmine and physiologic salt solution intravenously. Three-position films of the abdomen at first were not distinctive but

three days later were consistent with paralytic ileus. The white blood cell count was 22,400, with a polymorphonuclear count of 100. Urinary 17-ketosteroids were reported as 1.6 mg. in twenty-four hours.

The differential diagnosis seemed to rest between myxedema and, because of the low serum cholesterol level, panhypopituitarism. Despite DCA (5 mg. daily), ACTH (25 mg. four times daily), penicillin, streptomycin, Terramycin, gastric and colonic lavages, and intravenous saline, the patient became more distended, febrile and comatose. The serum sodium level was 136.4 mEq./L., potassium 3.3 mEq./L., nonprotein nitrogen 26 mg., per 100 ml., and sugar 112 mg. per 100 ml.

Four days after the onset of the abdominal complication, the suggestion was made that "myxedema ileus" was present (Bastenie; *Lancet* **1**: 413, 1946). Thyroid was started in a dosage of 0.015 Gm. daily. From that point on, the patient began to improve and left the hospital twenty-two days later, evidently on the road to complete recovery.

A week before discharge, a thyrotropin test to differentiate between primary myxedema and pituitary myxedema was performed. Pituitary thyrotropin was given for three days, in a dosage of 10 units intramuscularly daily. The 24-hour thyroidal uptake of I^{131} rose from 14 per cent before injection to 42 per cent after injection. The serum precipitable iodine (SPI) level was 2.9 gamma per 100 ml. before, and 7.6 gamma per 100 ml. after injection. This was a clear-cut response and tended strongly to confirm the diagnosis of pituitary disease.

The final discharge diagnoses were: panhypopituitarism (pituitary myxedema) of unknown etiology; eventration of the left diaphragm; and arteriosclerotic heart disease with ? Stokes-Adams syndrome.

The patient was well six months later, and was taking thyroid 0.12 Gm. daily, sodium chloride 5 Gm. daily, and cortisone 0.125 Gm. and 0.25 Gm. on alternate days.

DISCUSSION

The evidence for pituitary disease in this patient is strong. There was functional failure of both the thyroid and adrenal glands. Although adrenal failure can occur in primary myxedema because of the lack of thyroid hormone, considerably milder degrees of adrenal impairment are the rule. The low serum cholesterol level and the normal thyroidal uptake of I^{131}, associated with the markedly lowered BMR and SPI levels, were characteristic of panhypopituitarism rather than primary myxedema. Finally, the response of the thyroid to the administration of thyrotropin was that of an intact gland not functioning because of lack of endogenous thyrotopic stimulation, rather than that of a primarily diseased thyroid unable to respond even to the compensatory excess of endogenous thyrotropin which is known to be present under this circumstance.

The major and deceptive symptomatology which developed in the patient was the result essentially of hypothyroidism rather than of panhypopituitarism. Mental confusion and psychotic states associated with hypothyroidism have been recognized for years, and the syndrome has only recently again been given attention under the name "myxedema madness."

Syncopal spells despite prolongation of the electrocardiographic P-R interval, are a less well-recognized complication of myxedema. The patient

had arteriosclerotic heart disease, and the spells ceased during thyroid replacement therapy, suggesting that myxedema was the essential precipitating factor. Nevertheless, it is possible that the spells were of central nervous system origin and that heart disease played only an indirect role. With the onset of myxedema, both cardiac output and cerebral blood flow decrease; in combination with the cerebral arteriosclerotic change, this may have been enough to induce syncope.

The abdominal complications, culminating in paralytic ileus and unrelieved by adrenal replacement therapy, fitted well with the syndrome of myxedema ileus due to deposits of mucoprotein within the intestinal wall. The disappearance of the syndrome upon the administration of desiccated thyroid would seem to confirm this interpretation.

The loss of salt and water in a patient with adrenal insufficiency is not in itself surprising and is a recognized, although uncommon, overt feature of panhypopituitarism. However, the concomitant lack of increase in the serum potassium level was an odd feature in this patient, and the failure of the serum sodium level to respond to salt administration until potassium was given was even more unusual. A potassium deficit as well as a sodium deficit is implied; this parallels the situation seen in surgical patients with deficiencies of both electrolytes, but with intact adrenal function. Administration of physiologic saline alone results only in a continued diuresis of the salt and water.

Elevation of the spinal fluid protein content as seen in this patient, is a well recognized occurrence in myxedema. It is unusual, however, for the serum globulin level not to be increased, especially when the serum albumin level has decreased.

The cause of the pituitary disorder was not discovered. Panhypopituitarism in the male is almost invariably the consequence of destruction of the normal pituitary by tumor growth, particularly chromophobe adenoma. About 1 to 3 per cent of patients are seen before the sella turcica is expanded and hence no abnormalities are evident upon x-ray examination of the skull. Thrombosis of the blood vessels, as in postpartum necrosis of the pituitary, has not been described secondary to arteriosclerosis. However, such an event could perhaps have occurred in this patient. Tuberculosis also is a rare cause of pituitary destruction with an intact sella, but this possibility is unlikely in a man 58 years of age.

SUMMARY

A case report is presented of a 58-year-old male with pituitary myxedema of unknown origin. Onset was abrupt and associated with syncopal episodes and a state of mental confusion. Abdominal pain, distention and paralytic ileus developed in the later course of the disease. Progress was

steadily downhill despite vigorous adrenal replacement therapy, until thyroid replacement therapy was begun. At this point recovery began and continued until it was complete. Well-being was found to be maintained with treatment at the time of follow-up observation six months later.

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Editorial

THE CONTRIBUTION OF TRIIODOTHYRONINE TO THYROID PHYSIOLOGY

UNTIL recently the naturally occurring amino acid, *l*-thyroxine, was considered to be the most biologically active compound of the thyroid. All analogues or derivatives of thyroxine proved to be less active than thyroxine itself. This, plus the fact that almost all of the organic iodine in the blood appeared to be in the form of thyroxine, led to the concept that *l*-thyroxine was the active hormone of the thyroid. Recently Gross and Pitt-Rivers (1) and Roche *et al.* (2) described a new compound, *l*-triiodothyronine, present in thyroid tissue and in blood. In addition, Gross and Pitt-Rivers (3) observed that *l*-triiodothyronine was several times as active as *l*-thyroxine and suggested that it was the true thyroid hormone. Their findings have been amply confirmed by several workers (4, 5, 6). The writer reported that after the daily administration of *l*-triiodothyronine to patients with myxedema for one or two weeks, upon omission of the medication the protein-bound iodine in the blood disappears rapidly and reaches myxedema levels in from sixteen to thirty-six hours. This phenomenon is accompanied by a rapid drop in metabolism so that the basal metabolic rate reaches myxedema levels in from seven to nine days. This is in marked contrast to the slow disappearance of protein-bound iodine from the blood and to the slow drop in metabolism over a period of from fifty to seventy days after omission of thyroxine. These results suggest that triiodothyronine leaves the extracellular space rapidly to go into the tissue cells where it exerts its effect. Then, in turn, it is cleared rapidly from the intracellular fluid. Thyroxine could be considered the reservoir for triiodothyronine. The slow action of thyroxine and the time lag in the action of thyroxine may be due either to the slow degradation of thyroxine to triiodothyronine in the blood or tissues, or to the poor permeability of cells to thyroxine.

These findings support the concept of Gross and Pitt-Rivers that *l*-triiodothyronine is the active thyroid hormone. However, the report by Lipmann (7) that the effect on the "efficiency of respiration" of a mitochondrial system is greater with thyroxine than with triiodothyronine, although the oxygen consumption of such a system is greater with triiodothyronine, throws doubt on this theory. The increased oxygen consumption,

which is only one aspect of cell metabolism, may be due to the greater permeability of cells to triiodothyronine as compared to thyroxine. The small amounts of triiodothyronine may, therefore, be of no importance. This question cannot be settled at present. It is conceivable that the thyroid makes two hormones, each essential to the body economy.

It has been known for a long time that the greater portion of the iodine in thyroglobulin is accounted for by thyroxine and diiodotyrosine—about 30 per cent by thyroxine and 70 per cent by diiodotryrosine. The question as to what determines the potency of the thyroid hormone has puzzled us. Is it the thyroxine iodine or the total iodine? Since thyroglobulin as such does not exist in the blood stream (8) it must be broken down into smaller fragments before leaving the thyroid follicle. Since most of the hormone in the blood exists as thyroxine, it may be assumed that diiodotyrosine is either converted into thyroxine as the thyroglobulin is broken down, or is utilized to make new thyroglobulin. Earlier work with thyroxine and thyroglobulin led us to conclude (9) that the activity of thyroid hormone is determined by its total iodine content rather than by its thyroxine portion. It was assumed, therefore, that diiodotryosine is converted to thyroxine as the protein thyroglobulin is broken down.

Furthermore, at the time of the foregoing experiments the data suggested that *d*-thyroxine was as potent as *l*-thyroxine. By now it has become clear that *l*-thyroxine possesses all of the activity of a "*dl*" mixture. Consequently, we have reinvestigated this problem by treating several patients with myxedema with a purified thyroglobulin preparation, each dose containing 0.5 mg. of total iodine and 0.15 mg. of *l*-thyroxine iodine. In these patients the metabolic response was comparable to that produced by 0.25 mg. of iodine in the form of crystalline *l*-thyroxine. How do we resolve this discrepancy? One way is to assume that part of the diiodotyrosine is converted to thyroxine. The other and more likely way is to consider the contribution of triiodothyronine present in thyroglobulin. If triiodothyronine should constitute 3 or 4 per cent of the organic iodine of thyroglobulin, then it would contribute activity equal to 0.1 mg. of thyroxine iodine per dose. Consequently, the combined activity of *l*-triiodothyronine and *l*-thyroxine may account for the entire biologic activity of thyroglobulin. Actually Benua and Dobyns (10), in studying the radioactive compounds of the serum of 2 euthyroid patients following intensive radiation with radioactive iodine, found that triiodothyronine contributed 5 per cent of the total radioactivity.

If this be true, what happens to the diiodotyrosine present in thyroid hormone? The work of Roche *et al.* (11) has supplied a partial answer. Their finding of a deiodinating enzyme which breaks off iodine from diiodo-

tyrosine, but not from thyroxine, may actually account for the fate of diiodotyrosine.

The advent of triiodothyronine has resurrected Plummer's concept of "dysthyroidism." Plummer postulated that the "Graves' disease" gland secreted a hormone more toxic than the normal gland. In the past we have assayed myxedema patients with thyroglobulin derived from human "nontoxic glands" as well as from human "toxic glands." A re-evaluation of these data (12) suggests that the response to thyroglobulin derived from "toxic glands" is greater than the response to thyroglobulin from "nontoxic glands" given in equi-iodine dosage, in spite of the fact that the thyroxine content of the "toxic gland" is often lower than that of the "nontoxic gland." Perhaps the difference in activity may be explained by the amount of triiodothyronine present in each type of gland. This concept, also, is supported by the work of Benua and Dobyns (10), who found that the serum of thyrotoxic patients treated with radioactive iodine contains more radioactive triiodothyronine than the serum of euthyroid patients so treated. Furthermore, Freedberg and Hamolsky (13) showed that biologically labelled hormone in the serum of hyperthyroid patients treated with radioactive iodine has a greater disappearance rate than labelled hormone in the serum of treated euthyroid patients. They also suggested a possible qualitative difference in the nature of the hormone elaborated in various thyroid states. Future work may help explain these results and throw new light on Plummer's theory.

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota Medical School)

Answers by Samuel F. Haines, Mayo Clinic, Rochester, Minn.

QUESTION: What effect does climate have on thyroid function? What is the effect of hot weather and altitude on thyroid dosage?

ANSWER: Rats subjected to cold are reported to have hypertrophy of the thyroid. Gottschalk and Riggs (1) found no difference in the levels of serum protein-bound iodine or of the basal metabolic rates in 7 normal subjects before, during and after spending a few winter months in northern Canada. They did find that the serum protein-bound iodine level of a number of Eskimos was appreciably higher than that of normal subjects in Massachusetts. The basal metabolic rates of Eskimos have been reported by some to be higher than normal; however, Heinbecker (2), found the basal metabolic rates of Eskimos who were living on a mixed diet to be similar to those of persons living on such a diet in temperate zones.

I know of no evidence that weather or altitude affects the quantity of administered thyroid which is needed to hold the basal metabolic rates of myxedematous subjects at a normal level.

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QUESTION: Have you seen thyrotoxicosis of the exophthalmic type (*i.e.*, due to diffuse hyperplasia) in the presence of pre-existent panhypopituitarism?

ANSWER: Haines, MacLean and Craig reported in abstract a case of Graves' disease associated with a chromophobe pituitary tumor and concomitant evidences of anterior-pituitary insufficiency (*Proc. Central Soc. Clin. Research* **15:** 60–61, 1942). The presence of both lesions was proved at operation. The degree of anterior pituitary insufficiency in this case was questionable. Urinary assays for TSH were carried out some time after thyroidectomy and surgical removal of the pituitary tumor; by the methods then available, TSH was found to be present in the urine. The implications of the coincidental occurrence of these two diseases in this case are questionable.

QUESTION: In normal people who have a B.M.R. of −20 per cent, does administration of 2 or 3 grains of thyroid daily elevate the B.M.R.? At what B.M.R. level will the patient show evidence of toxicity?

ANSWER: The administration of 2 or 3 grains of desiccated thyroid (U.S.P.) to essentially normal people whose basal metabolic rates are in the neighborhood of −20 per cent

frequently increases the basal metabolic rate to average normal values. In some instances such doses will increase the basal metabolic rate to higher than average normal levels. Evidences of toxicity usually are not seen in these people until the basal metabolic rate has been increased to values above the average normal range; however, this is not consistently true, and it is considered that individual observation should determine the level to which the basal metabolic rate is raised in any particular case.

The foregoing statement is not intended to imply that the administration of desiccated thyroid to relatively normal persons who have low basal metabolic rates is necessarily a desirable procedure. In the great majority of such instances, there appears to be no adequate reason for this medication.

QUESTION: Is it ever necessary to exceed 3 grains of thyroid daily in the treatment of myxedema?

ANSWER: In my experience, the average dose of desiccated thyroid (U.S.P.) needed for the proper treatment of myxedema has been approximately 2 grains daily. Enough variation occurs in the individual response to desiccated thyroid so that the dose should be established for each patient on the basis of the clinical effect and the value of the basal metabolic rate, with particular consideration of the general clinical status of the patient. For example, it is not desirable to increase the basal metabolic rate of a patient who has myxedema and severe independent cardiac disease to as high a level as is desirable in a young and otherwise normal patient who has myxedema. Rarely have I found it necessary to use as much as 3 grains of desiccated thyroid daily for adequate treatment of myxedema, and I have exceeded that dose only in rare instances.

Patients in some other categories have required larger doses for elevation of the basal metabolic rate to the average normal range, but the desirability of increasing the basal metabolic rate in many patients in other categories is often questionable. The safety of such a procedure, particularly in patients who have anterior pituitary insufficiency and resultant myxedema, is much in question, unless previous treatment directed toward adrenal insufficiency is instituted.

Answers by Lawson Wilkins, Johns Hopkins Hospital, Baltimore, Md.

QUESTION: Please discuss the treatment of various types of dwarfism.

ANSWER: Hypothyroidism is the one type of dwarfism which is most amenable to treatment. In such cases, the usual dosage of desiccated thyroid is all that is required to bring about rapid and spectacular growth. Unfortunately, no preparations of anterior pituitary growth hormone have been found effective in the human being, although they are so in some other species. In spite of some claims, vitamin B_{12} and other vitamins have not proved to be of any value. Androgens, such as the various preparations of testosterone, stimulate protein anabolism and growth but this action is accompanied by virilizing effects. It has been claimed that methylandrostenediol would accelerate protein anabolism and growth without causing virilization. However, it now seems that this steroid is definitely a weak androgen and causes growth only when it is given in sufficient dosage to bring about some virilization. It probably has no place in therapy.

In primordial or genetic dwarfs who show relatively normal osseous development and normal sexual development, there is no indication for the use of androgens or other hormones. On the other hand, in hypopituitary dwarfs, who show marked osseous retardation and who remain sexually infantile after the age of 17 years, testosterone is of

great benefit and will bring about a spurt of growth simulating that of adolescence. In males, a testosterone preparation is usually the only treatment indicated. In females with hypopituitary dwarfism and sexual infantilism, estrogenic hormones should be given, at first continuously and then cyclically, accompanied by small doses of methyltestosterone (10 to 20 mg. per day). In females with ovarian agenesis and dwarfism there is usually relatively little delay in epiphyseal development, and not much increment of growth can be expected from treatment.

The treatment of constitutional delay in growth and delay in the onset of adolescence is open to debate. It is probably best not to begin therapy before a boy is 15 or 16 years of age unless his retardation causes serious psychologic problems. At this time, he may be treated with chorionic gonadotropin in order to accelerate the onset of puberty and the adolescent spurt of growth. Treatment should not be too intensive or too prolonged. It is probably safe to give from 1,000 to 2,000 units twice a week for a period of from three to six months.

QUESTION: Please differentiate between retardation of growth and delayed closure of epiphyses?

ANSWER: "Retardation of growth" refers merely to delay or stunting of linear growth (height); whereas "retardation of osseous development" indicates that there is delay in the time of ossification and/or the fusion of the epiphyses.

QUESTION: How can the anterior pituitary growth hormone be inhibited in order to prevent gigantism in adolescence, end especially to prevent girls from growing to a height 6 feet or over?

ANSWER: Excessive growth in adolescence resulting in tall stature is usually due to an inherited genetic trait and not to an endocrine disorder. Radiation of the pituitary is illogical and ineffective, except in patients with eosinophilic adenoma; these cases are exceedingly rare. If sufficient radiation could be given to damage the normal pituitary, its gonadotropic activities would probably be affected first. It has been suggested that estrogen be given to inhibit growth and hasten epiphyseal fusion. This would seem to be unwise during adolescence, because estrogen inhibits pituitary gonadotropic secretion and would interfere with the normal maturation of the pituitary-ovarian mechanism.

QUESTION: What is the mechanism underlying progeria?

ANSWER: The term "progeria" is often used loosely to describe an appearance of premature aging. It is generally applied to a peculiar type of dwarf described by Hutchinson and by Gilford. Cockayne described a different type of cachectic dwarf with microcephaly, retinal degeneration and pinched features, who had some progeric characteristics.

The cause of progeria is undetermined. One might surmise that there is some inborn error of metabolism which prevents the proper utilization of some unknown cellular component.

Answers by Laurance W. Kinsell, Highland Alameda County Hospital, Oakland, California

QUESTION: What are the indications for hypophysectomy?

ANSWER: Partial hypophysectomy is indicated in any patient with an hypophyseal tumor, 1) if the neoplasm is causing pressure effects on the gland itself (chromophobe

tumor), and/or 2) pressure effects on important structures close to the hypophysis, particularly the optic nerve (eosinophilic tumor), and 3) if the tumor is unresponsive to hormonal and/or radiation therapy.

Total hypophysectomy is now being performed experimentally in patients with advanced carcinomatosis and in patients with severe "malignant" diabetes mellitus. This procedure is too new to permit of critical evaluation.

QUESTION: What is the mechanism of the development of diabetes in acromegaly?

ANSWER: All pituitary growth-hormone preparations so far available, with one possible exception, have significant diabetogenic effects as well as growth-promoting effects. The pattern of diabetogenesis is somewhat different qualitatively from that which occurs when ACTH or cortisone is administered. Further, some of these growth-hormone preparations have been shown to be almost or completely free of ACTH contamination. One must, therefore, conclude that a hormonal factor, identical with growth hormone or closely associated with it in a physical-chemical sense, has a significant diabetogenic effect in experimental animals and in humans when administered in sufficient quantities over sufficient periods of time. The precise mechanism is undetermined. It is also unknown whether the diabetogenic material is actually an integral part of the growth hormone or whether it is a distinct factor, which may eventually be separated by appropriate chemical fractionation.

Excessive ACTH production may also play some part in the diabetes of many acromegalics, inasmuch as one frequently finds adrenal hyperplasia and small adrenal adenomata in these patients.

QUESTION: Does the serum phosphorus level invariably drop to normal after adequate x-ray irradiation of the pituitary in acromegaly? Would you repeat such therapy in a patient who eighteen months after x-ray therapy had no visual disturbances, no radiographic evidence of further destruction and no signs of clinical activity of the tumor, but a serum phosphorus level between 4.6 and 4.8 mg. per 100 ml. (highest pretreatment level, 5.1 mg. per 100 ml.)?

ANSWER: In our experience, the serum phosphorus level almost *never* drops to normal in response to radiation, and almost *always* drops to normal if one uses adequate hormonal therapy (estrogen and/or androgen). In the patient just referred to, I would suggest that hormonal therapy be instituted rather than further radiation therapy (1).

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QUESTION: What is the present-day attitude toward the diagnosis of pituitary headache? It has been frequently diagnosed and treated with x-ray therapy in the past.

ANSWER: To the best of my knowledge, "pituitary headache" does not exist as a specific entity. Many acromegalics with a large pituitary tumor have severe headaches, which may or may not respond to radiation therapy. It is thought that these headaches are referable to the production of actual tension in the dura, pia, and arachnoid by the expanding tumor.

QUESTION: Please discuss the use of cortisone during surgery for a pituitary tumor not associated with pituitary failure.

ANSWER: Removal of a pituitary tumor may result in major temporary or permanent damage to all functional aspects of the pituitary itself. Consequently, the patients are likely candidates for postoperative shock, on the basis of adrenal insufficiency. ACTH or cortisone, therefore, may be used to excellent advantage in the preoperative and postoperative management of such individuals. Large dosage is not required; a dose of 8 units of ACTH plus 50 mg. of cortisone per day should be ample in most instances.

Answers by C. L. Buxton, Yale University School of Medicine, New Haven, Conn.

QUESTION: Is there any advantage or better response to estrogen therapy if one uses a combination of natural and synthetic estrogens?

ANSWER: So far as we know, there has been no proof of any difference in estrogenic activity between natural estrogens and synthetic estrogens of the stilbestrol group. If this is true, there would be no particular reason for combining these two types of estrogenic hormones.

QUESTION: Please discuss the management of premenstrual mastalgia.

ANSWER: Many types of hormonal and other therapy have been used in the treatment of premenstrual mastalgia, none of which has been particularly successful. It is our impression that premenstrual mastalgia not infrequently occurs in individuals who are anovulatory. In any case it would be important to determine in each instance, by means of endometrial biopsy, whether or not the patient were ovulating. If the patient were not ovulating, it would seem reasonable to try replacement of the progesterone which would be missing from the circulation premenstrually because of anovulation. Aside from this, and symptomatic therapy, we have no useful suggestions to offer.

QUESTION: What is the explanation for decidual casts at the time of menstruation? Are such casts found frequently in sterility studies?

ANSWER: Although decidual casts are described in most text books we have actually never seen one, except in cases of ectopic pregnancy in which the decidua was discarded during the process of trophoblastic disintegration in the tube.

QUESTION. When you have found on endometrial biopsy that a patient has a progesterone deficiency causing an impairment in the secretory phase, and the patient becomes pregnant, do you believe it necessary to administer progesterone during the first trimester in order to prevent early abortion?

ANSWER: If a patient has a real progesterone deficiency which impairs development of secretory endometrium, the probabilities are that she will not become pregnant. If, by any chance, she does become pregnant, it is probable that the chorionic gonadotropin produced by the trophoblast will stimulate sufficient progesterone production to supply the needs of the first trimester of pregnancy.

WILLARD O. THOMPSON M.D.

1899–1954

LIFE came to a sudden close for Dr. Willard O. Thompson on March 23, 1954, culminating a brilliant medical career. In this untimely death the medical profession lost one who gave unselfishly of his time and efforts for the betterment of medicine and mankind.

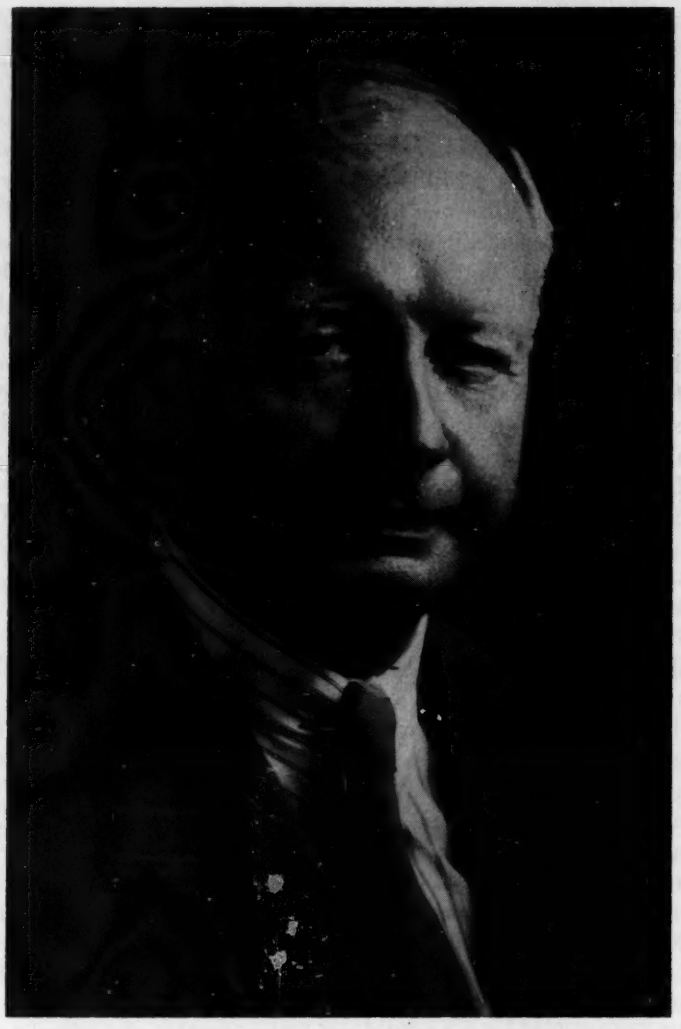
Dr. Thompson was born in Fredericton, New Brunswick, Canada, on February 17, 1899. In 1919 he received his B.A. degree from Dalhousie University, Halifax, Nova Scotia, and in 1923 his M.D. degree from Harvard Medical School. After his internship at the Boston City Hospital, he was a Fellow of the National Research Council and held various Research Fellowships in medicine at Harvard Medical School and the Massachusetts General Hospital, where he carried on his investigative work in the Thyroid Clinic under Dr. J. H. Means.

In 1929 he left Boston and joined the faculty of Rush Medical College as Assistant Clinical Professor of Medicine. In 1935 he was made Associate Clinical Professor. From 1941 until the time of his death, he was Clinical Professor of Medicine at the University of Illinois College of Medicine. From 1930 to 1946 he was Attending Physician at the Presbyterian Hospital, Chicago; and after 1946, Attending Physician at Grant, Henrotin, and Illinois Research Hospitals.

For his outstanding efforts many honors and positions were given him and many of these called on his will to public service. He was President of the American Geriatrics Society; Past-President of the American Goiter Association, of the Mississippi Valley Medical Society, and of the Chicago Medical Society; and a former Vice-President and Council member of the Endocrine Society. He was a Fellow of the American College of Physicians and a member of the Association of American Physicians, the American Society for Clinical Investigation, the Central Society for Clinical Research, the American Therapeutic Society, the Gerontological Society, and the American Diabetes Association.

Dr. Thompson's notable genius for the organization of medical meetings and educational assemblies was in constant use by his fellows. In 1945 the Commanding General of the Sixth Service Command awarded him a Citation of Distinguished Service for his "untiring efforts and devotion" as Chairman of District No. 14, Wartime Graduate Medical Meetings. In 1952 he received the Distinguished Service Award from the Mississippi Valley Medical Society.

For many years he served as managing editor of this journal and served



WILLARD O. THOMPSON, M.D.

1899–1954

it well. He constantly sought a broader scope of coverage and the inclusion of papers from the Goiter Society and the development of editorials and teaching clinics are in great part due to him. He likewise edited the Journal of the American Geriatrics Society and the American Lectures in Endocrinology. He was a former editor of the Yearbook of Endocrinology.

His scientific contributions form a long list. Between 1926, when his first paper appeared, and 1937 his major effort was in the field of thyroid disease. He discovered that amounts of iodine, at the time supposed minute, controlled Graves' disease, thus bringing this influence within physiologic comprehensibility. He provided the only extant account of the life history of Graves' disease treated by iodine alone. A curious discovery that myxedema could be induced by iodine is still not sufficiently understood or developed. During the period 1930–37 he worked on the systematic measurement of the effects of diverse thyroid moieties on heat production and the course of myxedema. In 1936 and 1938 he provided an early and still standard description of the stimulation of the human thyroid by thyrotropin. From 1937 on he contributed greatly to our knowledge of the influences of chorionic gonadotropin on the testes and described both the sexual and the somatic aspects of this stimulation. In his later years much of this work and much not mentioned were incorporated, together with the products of a vast clinical experience, into reviews, articles and teaching clinics for the instruction of both his own students and the profession at large.

Everyone who came to know Willard Thompson was aware of his thoroughness and sincerity. No task was too difficult or time-consuming. His devoted patients found him a person of great understanding, who always took time to deal with their personal problems. He followed the words of Benjamin Franklin, who wrote in his "Poor Richard's Almanac" —'Dost thou like life? . . . then do not squander time for this is the stuff life is made of.'

His wife, Dr. Phebe K. Thompson (associated with him in his work since 1925), three sons and a daughter survive him.

NORRIS J. HECKEL

The Endocrine Society

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held in the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, in in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 18, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Hotel reservations may be made through the San Francisco Convention and Visitors' Bureau, Room 300, Civic Auditorium, San Francisco, California. They should be accompanied by the official application form which has appeared weekly in the J.A.M.A. since December 1953.

Arrangements have been made for members of this society who do not belong to the AMA to be assigned rooms during the days of the meeting of the Endocrine Society. Applications from nonmembers of the AMA should specify dates of arrival and departure and be accompanied by a deposit of $10.00 per room.

(For program, see April issue of the Journal.)

THIRD PAN-AMERICAN CONGRESS IN ENDOCRINOLOGY

SANTIAGO, CHILE

November 21–27, 1954

Members of The Endocrine Society are cordially invited to attend the Third Pan-American Congress in Endocrinology. The faculty is composed of eminent investigators in endocrinology from North and South America.

This is an unusual opportunity for you and your family to enjoy a pleasant vacation in South America and for you to attend a highly instructive medical assembly. Correspondence should be directed to Dr. Arturo Atria, Hospital del Salvador, Casilla 70-D, Santiago, Chile. A copy of the tentative program may be obtained from the Secretary-Treasurer, The Endocrine Society, Suite 319, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

THE UNITED STATES PHARMACOPŒIAL CONVENTION INC., 1950–1960

Steroid Reference Substances

The U.S.P. Board of Trustees has announced a new U.S.P. service made possible through cooperation with the National Institutes of Health of the U. S. Public Health Service. New steroid substances have been prepared under the direction of the U.S.P. Steroid Advisory Board and will be distributed by authority of the U.S.P. Board of Trustees.

The tremendous advances in the field of endocrinology have focussed attention on the complex steroid substances which are now known to play a vital role in human physiology, both normal and pathologic. The identification of steroid excretion products is highly essential in the diagnosis of certain forms of cancer and, furthermore, certain steroids are important therapeutic agents. Many steroids are rare and available from few sources, yet only minute quantities of them are invaluable for purposes of identification in paper chromatography and infrared spectrophotometry. Thus the Endocrinology Study Section of the National Institutes of Health initiated work which eventually led to the formation of the U.S.P. Steroid Advisory Board and the collection of a first set of U.S.P. Steroid Substances.

The first set comprises 24 steroids, and additional sets will become available as soon as the necessary steroids can be obtained and checked.

It is believed that the availability of these steroids for the purposes stated will be heartily welcomed by endocrinologists both in the clinic and in the laboratory.

In instituting this new service, the U.S.P. Board of Trustees is grateful for the opportunity to make greater use of the well-established distribution facilities by which 52 U.S.P. Reference Standards are provided at cost for pharmaceutical control and research not only within the United States but to all parts of the world.

The price is $3.00 for a single steroid and $60.00 for the complete set of 24. All requests should be directed to, and checks made payable to: U.S.P. Reference Standards, 46 Park Avenue, New York 16, N. Y.

BOOKS RECEIVED

American Lecture Series. Charles C Thomas, Publisher, Springfield, Illinois

Acute Renal Failure. By ARTHUR GROLLMAN, PH.D., M.D., Professor and Chairman, Dept. of Experimental Medicine, Southwestern Medical School of the University of Texas, Dallas, Texas. A monograph in Am. Lectures in Internal Medicine, edited by ROSCOE L. PULLEN, M.D.; 96 pages; 6 illust.; 1954.

(*The*) *Adaptive Chin.* By E. LLOYD DUBRUL, M.S., D.D.S., Asst. Prof. in Charge of Oral Anatomy, College of Dentistry, Univ. of Illinois, and HARRY SICHER, M.D., D.Sc., Prof. of Anatomy and Head of the Department, Loyola Univ. School of Dentistry, Chicago, Ill. A monograph in Am. Lectures in Anatomy, edited by OTTO F. KAMPMEIER, M.D.; 105 pages; 47 illust.; 1954.

Chronic Iliac Pain in Women. By H. B. ATLEE, M.D., Head of Dept. of Obstetrics and Gynecology, Dalhousie University Medical School, Halifax, Nova Scotia, Canada. A monograph in Am. Lectures in Gynecology and Obstetrics, edited by E. C. HAMBLEN, M.D.; 70 pages; 6 illust.; 1954.

Cutaneous Manifestations of Systemic Diseases. By JOHN GODWIN DOWNING, M.D., Prof. of Dermatology and Syphilology, Tufts College Medical School, Prof. of Dermatology, Boston Univ. School of Medicine, Physician-in-Chief, Diseases of the Skin, Boston City Hospital, Boston, Mass. A monograph in Am. Lectures in Dermatology, edited by ARTHUR C. CURTIS, M.D.; 152 pages; 52 illust.; 1954. Price $4.25.

Dizziness: An Evaluation and Classification. By DAVID DOWNS DEWEESE, M.D., Clin. Prof. of Otolaryngology, Univ. of Oregon Medical School, and Head of Dept. of Otolaryngology, The Portland Clinic, Portland, Oregon. A monograph in Am. Lectures in Internal Medicine, edited by ROSCOE L. PULLEN, M.D.; 96 pages; 2 illust.; 1954. Price $2.75.

Menorrhalgia: Menstrual Distress. By WILLIAM BICKERS, M.D., Attending Gynecologist to Retreat for Sick, Sheltering Arms, Richmond Community and Evangeline Booth Hospitals, Richmond, Virginia. A monograph in Am. Lectures in Gynecology and Obstetrics, edited by E. C. HAMBLEN, M.D.; 104 pages; 11 illust.; 1954. Price $2.75.

Physiological Cardiology. By ARTHUR RUSKIN, M.D., Assoc. Prof. of Internal Medicine, University of Texas, Medical Branch, Galveston, Texas. A monograph in the Bannerstone Division of Am. Lectures in Physiology, edited by ROBERT F. PITTS, M.D.; 318 pages; 12 illust.; 1953.

Atlas of Exfoliative Cytology. By GEORGE N. PAPANICOLAOU, M.D. PH.D., Clin. Prof. of Anatomy, Emeritus, Cornell Univ. Medical College, New York. There are 4 figures in black and white. Of the 36 colored plates, 12 are devoted to the female genital system, 4 to the urinary and male genital organs, 5 to the respiratory system, 5 to the alimentary tract, 2 to the pleural, peritoneal and pericardial exudates, 2 to breast secretions, 1 to histiocytic cells, 1 to cells related to pregnancy, 1 to cells affected by irradiation, 1 to multinucleated cells of various types, and 2 to mitotic figures found in normal and malignant cases.

Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass. Loose-leaf, fabricoid. Price $18.00.

Clinical Endocrinology. An integrated account of the endocrine system, by KARL E. PASCHKIS, M.D., Assoc. Prof. of Medicine, Asst. Prof. of Physiology, Jefferson Medical College, Chief of Endocrine Clinic, Jefferson Medical College Hospital, Philadelphia, Pa.; ABRAHAM E. RAKOFF, M.D., Clin. Prof. of Obstetric and Gynecologic Endocrinology, Jefferson Medical College; and ABRAHAM CANTAROW, M.D., Prof. of Biochemistry, Jefferson Medical College; 842 pages; 253 illust., 5 in full color; 1954. Paul B. Hoeber, Inc., New York, N. Y. Price $16.00.

Parental Age and Characteristics of the Offspring. Twenty-two contributors. Editor R. W. MINER; Consulting Editor, L. C. STRONG. Article 5 (pages 451–614) from the Annals of the New York Academy of Sciences, Volume 57, January 15, 1954. This series of papers is the result of a Conference held by the Section of Biology of the Academy, January 23 and 24, 1953.

Studies in Schizophrenia. A Multidisciplinary Approach to Mind-Brain Relationships. A report of the development and testing of a new theory of schizophrenia by the TULANE DEPT. OF PSYCHIATRY & NEUROLOGY, reported by ROBERT G. HEATH, Chairman; 633 pages; illustrated; 1954. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass. Price $8.50.

Transactions of the American Goiter Association for 1953. A collection of papers dealing with the latest clinical and physiologic advances in the study of the thyroid gland, by outstanding authors in the field; 437 pages; illustrated; 1954. Charles C Thomas, Publisher, Springfield, Illinois. Price $13.50.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Sales Office, P.O. Box 575, Packanack Lake, New Jersey.

Administrative Medicine. First Conference, March 9, 10 and 11, 1953, New York, N. Y. Edited by GEORGE S. STEVENSON, M.D., Medical Director, National Association for Mental Health, New York; 180 pages; 1953. Price $3.00.

Cold Injury. Second Conference, Nov. 20 and 21, 1952. Edited by M. IRENÉ FERRER, M.D., Asst. Prof. of Clin. Medicine, Columbia Univ. College of Physicians and Surgeons, New York; 242 pages; illustrated; 1954. Price $4.00.

Connective Tissues. Fourth Conference, Feb. 18, 19 and 20, 1953. Edited by CHARLES RAGAN, M.D., Assoc. Prof. of Medicine, Columbia Univ. College of Physicians and Surgeons, New York; 197 pages; 1953. Price $3.75.

Nerve Impulse. Fourth Conference, March 4, 5 and 6, 1953. Edited by DAVID NACHMANSOHN, M.D., Assoc. Prof. Neurology, Columbia Univ. College of Physicians and Surgeons, New York; 224 pages; 1954. Price $4.00.